“A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT”

Dissertation Submitted in

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(BRANCH XX)

MADRAS MEDICAL COLLEGE

THE TAMIL NADU DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2016

CERTIFICATE

Certified that this dissertation titled **"A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT"** is a bonafide work done by **Dr.SHANMUGA PRIYA. K,** Postgraduate student of the Department of Dermatology, Venereology and Leprosy, Madras Medical College, Chennai – 3 during the academic year 2013 – 2016. This work has not previously formed the basis for award of any degree.

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DECLARATION

I **Dr. SHANMUGA PRIYA. K** solemnly declare that the dissertation on **"A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT"** was done by me at Madras Medical College during 2013-2016 under the guidance and supervision of **Prof**. **Dr.S. KALAIVANI, M.D., D.V.** Director in charge and Professor, Institute of Venereology, Madras Medical College/RGGGH, Chennai- 600003.

The dissertation is submitted to the Tamil Nadu DR.MGR Medical University towards the partial fulfillment of the rules and regulations for the award of **M.D Degree in Dermatology, Venereology and Leprosy (BRANCH – XX).**

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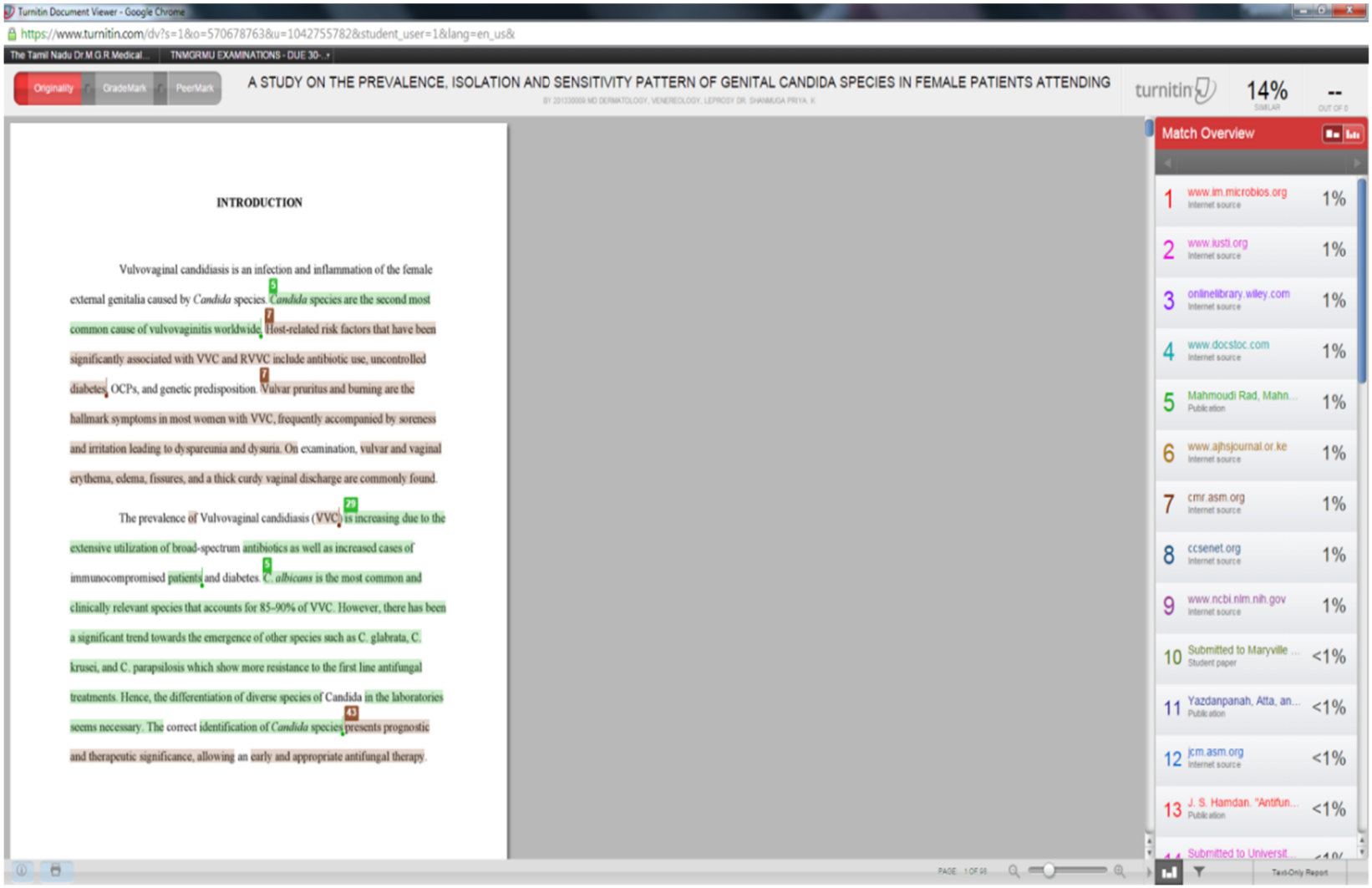
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INTRODUCTION
Vulvovaginal candidiasis is an infection and inflammation of the female external genitalia caused by Candida species. Candida species are the second most common cause of vulvovaginitis worldwide. Host-related risk factors that have been significantly associated with VVC and RVVC include antibiotic use, uncontrolled diabetes, OCPs, and genetic predisposition. Vulvar pruritus and burning are the hallmark symptoms in most women with VVC, frequently accompanied by soreness and irritation leading to dyspareunia and dysuria. On examination, vulvar and vaginal erythema, edema, fissures, and a thick curdy vaginal discharge are commonly found.
The prevalence of Vulvovaginal candidiasis (VVC) is increasing due to the extensive utilization of broad-spectrum antibiotics as well as increased cases of immunocompromised patients and diabetes. C. albicans is the most common and clinically relevant species that accounts for 85-90% of VVC. However, there has been a significant trend towards the emergence of other species such as C. glabrata, C. krusei, and C. parapsilosis which show more resistance to the first line antifungal treatments. Hence, the differentiation of diverse species of Candida in the laboratories seems necessary. The correct identification of Candida species presents prognostic and therapeutic significance, allowing an early and appropriate antifungal therapy.
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CONTENTS

"A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT"

ABSTRACT

INTRODUCTION

Vulvovaginal candidiasis is an infection and inflammation of the female external genitalia caused by *Candida* species. The prevalence of Vulvovaginal candidiasis (VVC) is increasing due to the extensive utilization of broad-spectrum antibiotics as well as increased incidence of immune compromised states and diabetes. There has been a significant trend towards the emergence of other species such as C. glabrata, identification of which has prognostic and therapeutic significance.

AIMS AND OBJECTIVES

1. To study the prevalence of various Candida species in female patients with vaginal discharge.
2. To study the susceptibility pattern of Candida to commonly used antifungals.

MATERIALS AND METHODS

It is a Prospective observational study of 200 female patients attending the STD OPD with or without symptoms. Patients aged <18 yrs and >60 yrs, pregnant, lactating & menstruating women, those not willing to participate in the study and those who had used antifungals within past 7 days were excluded. A detailed history was taken and complete genital examination was done. A sample of vaginal discharge was collected. Microscopic examination with Gram's stain and KOH and culture with Sabouraud's Dextrose Agar was done. Speciation was done using Chromogenic agar. Antifungal susceptibility was tested by disk diffusion method.

OBSERVATION AND RESULTS

The prevalence of Vulvovaginal Candidiasis proven by either culture or microscopy was 29%. Only 7% patients had pseudohyphae and spores on microscopic examination. Of the asymptomatic patients, 28.3% had VVC. C.glabrata (61.22%) was the most common species isolated followed by C.albicans (20.4%). Nystatin was the most effective antifungal (81.63%) followed by Miconazole and Fluconazole.

CONCLUSION

This study shows that if microscopy, which is the commonly used bedside test to confirm Candidiasis, alone is used for diagnosis, most of the VVC cases would be missed. Culture has significantly increased the detection of VVC cases. Culture and microscopy used in combination would be better than either tests used alone. This study has also proven the importance of considering Candida species other than C. albicans and drug resistance to first line antifungals as a cause of treatment failure.

KEYWORDS:

Vulvovaginal candidiasis, Antifungals, CHROM agar, C.glabrata.

INTRODUCTION

Vulvovaginal candidiasis is an infection and inflammation of the female external genitalia caused by *Candida* species. *Candida* species are the second most common cause of vulvovaginitis worldwide. Host-related risk factors that have been significantly associated with VVC and RVVC include antibiotic use, uncontrolled diabetes, OCPs, and genetic predisposition. Vulvar pruritus and burning are the hallmark symptoms in most women with VVC, frequently accompanied by soreness and irritation leading to dyspareunia and dysuria. On examination, vulvar and vaginal erythema, edema, fissures, and a thick curdy vaginal discharge are commonly found.

The prevalence of Vulvovaginal candidiasis (VVC) is increasing due to the extensive utilization of broad-spectrum antibiotics as well as increased cases of immunocompromised patients and diabetes. *C. albicans* is the most common and clinically relevant species that accounts for 85–90% of VVC. However, there has been a significant trend towards the emergence of other species such as C. glabrata, C. krusei, and C. parapsilosis which show more resistance to the first line antifungal treatments. Hence, the differentiation of diverse species of Candida in the laboratories seems necessary. The correct identification of *Candida* species presents prognostic and therapeutic significance, allowing an early and appropriate antifungal therapy.

Review of literature

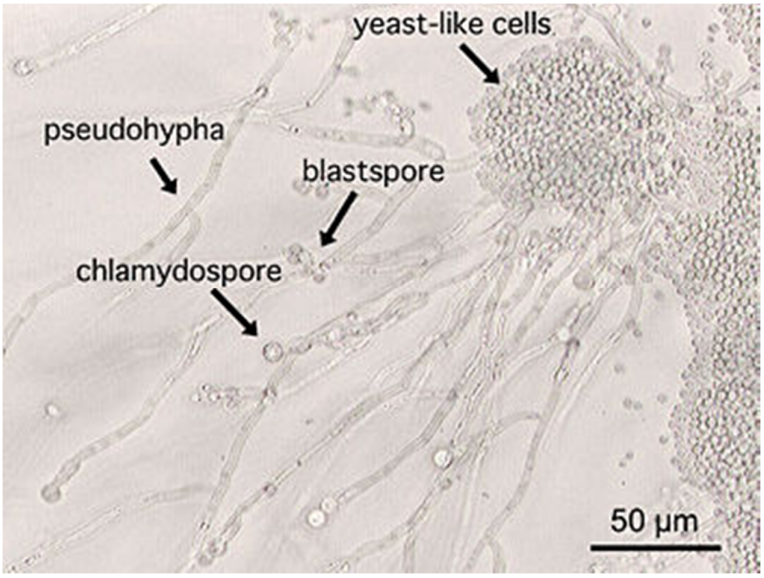
REVIEW OF LITERATURE

Vulvo vaginal candidiasis is the infection of female genital tract caused by the yeast Candida. It was first described in 1849 and the pathogen was named oidium albicans. Most common species causing VVC is Candida albicans. Many other species are implicated now and among that Candida glabrata is responsible for most of the infections next to C. albicans, especially in Asian countries.

MYCOLOGY

C. albicans is yeast of size 2-6 µm. It belongs to the division Ascomycota and family Saccharomycetales. It is a dimorphic fungus i.e.,it has an ability to grow in two different ways; reproduction by budding in hyphal form or in yeast-like forms. Therefore it produces different morphological forms under different environmental conditions such as,

- Budding yeast cells (blastospores, blastoconidia)
- Pseudohyphae (elongated cells which appear as filamentous cell chains)
- True hyphae
- Chlamydospores.



Blastospores are unicellular forms of the fungus that divide by budding. Budding is the growth of new cell from the blastospore surface. Nuclear division follows and a septum is laid down between the parent and daughter cell units. The two cell units then separate to form individual blastospores. Some environmental factors, favour a cylindrical outgrowth on the surface of a blastospore which forms the germ tube. Germ tubes grow continuously by extension and mitotic cell division occurs within the extending tube. Septa are formed at intervals in the extending apical tip to form a hypha. A hypha is a long tubular structure comprising multiple fungal cells which are divided by septa. New hyphae arise as branches from existing hyphae or by germination of spores. A mycelium is an entire fungal cellular aggregate that includes hyphae with all their branches. Spores that form on the pseudohyphae are called chlamydospores.

The main factors that favour yeast to hyphae transformation are Temperature 35°C (hypha with higher temperature)

- pH 7.0 (hypha with higher pH)
- Initial blastospore concentration not exceeding 10^6/ml
- Presence of different compounds, such as N-acetylglucosamine, proline, amino acids, biotin, sulfhydryl groups, heme, zinc and serum.

The most critical factor for in vivo induction of the mycelial form is serum or macrophages[28].

The ability of the organism to transform between the yeast and the hyphal forms has been implicated in its pathogenicity[28].

C. albicans lacks a sexual cycle and is a diploid organism. The cell wall of Candida is multilayered located outside the plasma membrane. It is composed of different types of carbohydrates (80 – 90 %):

(i) Mannan or mannoproteins (mannans with glycoproteins)

(ii) Į-glucans - polymers of glucose (branched)

(iii) Chitin - polymer of N-acetyl-D-glucosamine (un-branched)

The other constituents are proteins (6 - 25 %) and lipids (1 – 7 %). Yeast cells and germ tubes have similar cell wall composition with varying amounts of Į-glucans, chitin and mannan. Although glucans are the major constituents in C. albicans, they are immunologically less active.

C. albicans is a constituent of the normal human flora present in the skin, gastrointestinal and genitourinary tracts. It colonizes mucosal surfaces of the oral and vaginal cavity and can cause a variety of infections, in the presence of a defect in host immune system. Candidiasis may be divided into superficial (such as mucocutaneous candidiasis) and deep-seated (such as *Candida* septicaemia).

The genus *Candida* comprises many different species of which *C. albicans* is considered the most common and important. However, in recent years, other species, such as *C. krusei*, *C. glabrata*, C. parapsilosis, C.*tropicalis*, C. *guilliermondi*, C. *Kefyr* and C. *cerevisae* have also been introduced as important pathogens.

PATHOGENESIS

The first step in pathogenesis of VVC is the adherence of candida to host epithelial cells. C. albicans has the greatest affinity for vaginal epithelial cells followed by C. tropicalis and C. parapsilosis. Hypha forms a biofilm layer that strongly adheres to the vaginal epithelium. Factors influencing adherence include nutrients like glucose, estrogen, IgA, fibronectin, candida cell surface hydrophobicity and mannoproteins.

Adhesion is followed by invasion which is favoured by certain enzymes produced by candida like proteinases, phospholipases and hyaluronidases. Proteinases break down peptide bonds and aspartyl proteinases are

collagenolytic. These enzymes also break down local immunoglobulins. Hyphae along with the enzymes aid in invasion.

Virulence factors of candida include SAP (secreted aspartyl proteinases), Factor H, plasminogen binding mannoprotein complexes of the cell wall and Hypha-associated proteins. SAP has the capacity to enzymatically degrade tissue barrier proteins (e.g. E-cadherin) and other factors like complement and antibodies. Factor H and plasminogenbinding mannoprotein complexes of the cell wall interfere with function of complements. Hypha-associated proteins inhibit phagocytosis of Candida. Other factors inhibit cytokine production and/ or function[12].

HOST DEFENCE MECHANISMS

Integrity of mucosa is the first important defence against invasion. Normal vaginal flora with stable pH is protective against candidal infection. Macrophages and neutrophils are important in protection against systemic invasive candidiasis.

Cell-mediated immunity (CMI) is an important host defense against recurrent vulvo vaginal Candidiasis. Local expression of CMI in the vaginal mucosa is more important in this aspect. Vaginal mucosa possesses unique tissue-specific T cells distinct from peripheral Tcells[9]. Cytokines like IL-1b and IL-18 and lymphocyte subsets T-helper 1 and T-helper 17 have a role in protection against Candida. Conversion of C. albicans from vaginal commensal to pathogen results from abnormalities in CMI[9].

Humoral immunity is usually restricted to anti-Candida antibodies of the IgA class. Circulating IgA antibody is of high molecular weight and contains secretary component (secretary IgA). Secretary IgA is also present in cervicovaginal secretions which confirm the importance of local stimulation and regional response in mucocutaneous infections[11]. Women with elevated IgE levels are shown to have increased levels of Prostaglandin E2 in vaginal secretions which enhance germ tube production.

CLINICAL FEATURES:

VVC is the second most common cause of vaginitis in tropics next to bacterial vaginosis. 70 to 75% women will have atleast one lifetime episode and up to 90% is caused by C. albicans. VVC is classified into uncomplicated and complicated types.

Uncomplicated VVC:

- Sporadic or infrequent
- Mild to moderate
- Mostly caused by C.albicans
- Seen in females with intact immunity.

Complicated VVC:

- Recurrent VVC
- Severe VVC
- Caused by non C.albicans species

- Women with risk factors like uncontrolled diabetes, debilitation, immunosuppression or pregnancy.

Predisposing factors for candidiasis include,

1. Antecedent broad spectrum antibiotic use
2. Uncontrolled diabetes mellitus
3. Immunosuppression due to HIV
4. High dose estrogen oral contraceptive pill
5. Hormone replacement therapy
6. Pregnancy especially third trimester
7. Steroid intake
8. Douching
9. Perfumed hygiene products
10. Tamoxifen therapy
11. Occlusion with non cotton tight undergarments
12. Sexual activity
13. Sponges and IUCDs[22]

Antibiotic use leads to loss of normal vaginal flora. Uncontrolled diabetes leads to increased glucose levels which favours yeast growth. Steroids interfere with neutrophil phagocytosis.

Estrogen increases the formation glycogen in vaginal epithelial cells. Glycogen serves as the carbon source of Candida and increases its growth. It increases adherence of Candida to epithelial cells and enhances the formation

of mycelium. Th17 immune response against C. albicans is impaired. High dose estrogen oral contraceptive pill, hormone replacement therapy and pregnancy especially third trimester are associated with increased estrogen levels.

Other probable factors include

1. Iron supplements - unbound iron has been found to enhance C.albicans growth
2. Deficiency of vitamin A - impaired keratinisation
3. Protein deficiency – impaired host defences
4. Zinc deficiency
5. Atopy with skin test positivity to inhalant allergens

Symptoms:

- Pruritis – more severe at night
- Thick curdy white discharge
- Soreness and redness of vulva
- Dysuria
- Dyspareunia
- 10 – 20% are asymptomatic

Signs

- Vulvar erythema and swelling
- Maceration and soddening
- Fissures and erosions – in the vulva and vaginal mucosa

- Excoriation marks
- Visible adherent white discharge at the vestibule, introitus or vaginal mucosa (cottage cheese appearance)
- Inflamed vaginal mucosa
- Rash with satellite pustules
- Vestibulitis – tenderness in 3 and 9 o clock position around hymen when checked with a cotton swab
- Periurethral inflammation
- Watery or purulent discharge occasionally
- Perianal redness and fissures
- Intertrigo groin or gluteal cleft[36]

RECURRENT VULVOVAGINAL CANDIDIASIS (RVVC):

RVVC is a much more serious clinical condition with recurrence of symptoms (four or more episodes per year) and is refractory to treatment. It occurs in about 5% women. Few of the recurrences may be because of the persistence of the predisposing factors that underlie VVC. Most of the recurrences in RVVC are due to same strain due to the yeast residing in protected sites. But in the majority of cases it is idiopathic, as it occurs in women without any known risk factors. Genetic predisposition involving certain genes and an interaction with environmental factors has been implicated.

Single nucleotide polymorphisms (SNP) in genes coding for mannose-binding lectin, interleukin (IL)-4, Dectin-1 receptor, CARD 9, IL-22 and the enzyme indoleamine 2,3-dioxygenase in regulatory T-cells can predispose to RVVC. There is also evidence of partial T – cell dysregulation and high gamma interferon production, which is exacerbated during elevated estrogen levels in follicular phase of menstrual cycle. Non secretors of Lewis blood group have greater risk of RVVC. Anti-SAP antibodies are found in the vaginal secretions and blood of patients with RVVC.

LABORATORY INVESTIGATIONS FOR IDENTIFICATION OF CANDIDA

pH MEASUREMENT:

The pH of the vaginal discharge is low between 3 and 4.5. It is identified by taking swabs from the lateral vaginal wall and placing it over the pH paper. Contamination by blood, semen, cervical secretions and topical medications can affect the results.

MICROSCOPIC EXAMINATION:

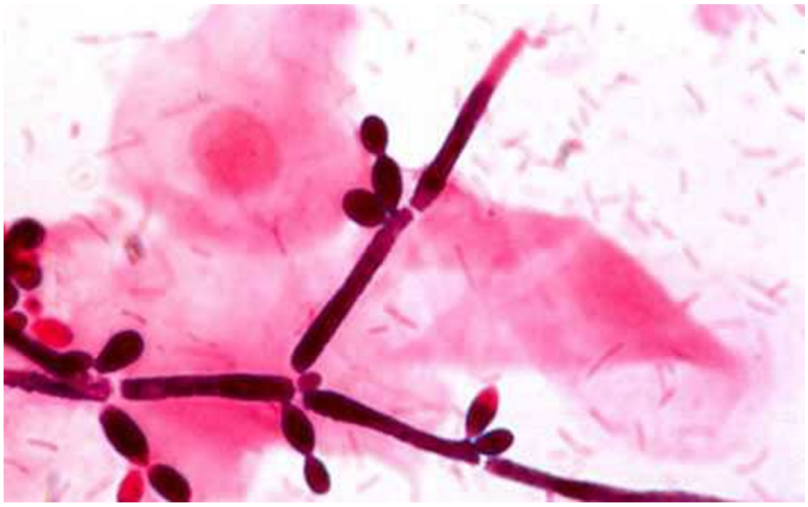
WET MOUNT:

A sample of vaginal secretion is taken with a loop and mixed with saline on one slide and with a drop of 10% KOH on another slide. Cover slips are placed and the slide is viewed under microscope.

Budding yeasts (blastospores) and pseudohyphae are seen. Clue cells and trichomonas can be ruled out. It has a sensitivity of 40 – 60%.

GRAM STAIN:

A swab containing vaginal discharge taken from the lateral walls of vagina is smeared on a glass slide. It is allowed to air dry and stained by Gram's technique. It is viewed under high power or oil immersion. Candida organisms are seen as gram positive hyphae and spores.



PAPANICOLAOU STAIN:

A cervical smear is taken and stained by papanicolaou stain. The smear shows marked inflammation associated with symptomatic disease. Candidal elements are difficult to identify in Papanicolaou -stained smears. The proportion positive by Gram stain is significantly greater than the proportion positive by Papanicolaou stain[43].

CULTURE:

Swabs are taken from the lateral vaginal wall and placed on Amie's transport medium or plated directly on Sabouraud's dextrose agar plate.

Composition of SDA is,

- Agar – 2%
- Dextrose – 4%
- Peptone – 1%
- pH – 5.6 ± 0.2 at 25°C

Peptone provides the nitrogenous compounds. Dextrose is the source of energy. Candida grows as shiny, cream coloured, yeasty smelling and smooth surfaced colonies.

Cultures are necessary

- To confirm the diagnosis
- When non C.ablicans species are suspected
- When antifungal susceptibility testing is needed
- In invasive candidiasis

Majority of symptomatic patients have more than 10^3 blastospores per ml and can be identified by culture of vaginal secretions. In those with low counts due to treatment or with disease due to hypersensitivity to candida antigen, culture results can be improved by taking vaginal washings or direct inoculation into broth media with antibiotics.

POLYMERASE CHAIN REACTION:

It is a highly sensitive molecular method for diagnosis of Candidiasis. Its use is limited in diagnosis of uncomplicated VVC. It is useful in diagnosing asymptomatic RVVC, species identification and invasive Candidiasis. The detection of candidal DNA is done by amplification of the small subunit rRNA gene, lanosterol demethylase gene, 5.8S rRNA gene including the adjacent nontranscribed spacer region, and the noncoding internal transcribed spacer (ITS) region of rRNA genes[48].

Sterile vaginal sample is obtained and Candida DNA extracted. The PCR mixture consists of primers, template DNA, Taq DNA polymerase and PCR buffer

The PCR mixture is amplified using the following conditions:

- Denaturation
- Annealing
- Extension
- Final extension

1.2% agarose gel electrophoreses is performed. The gel is pre-stained with 0.05% ethidium bromide. The DNA bands are detected by ultraviolet transilluminator[57].

SPECIES IDENTIFICATION:

Species can be identified based on

- Culture characteristics
- Biochemical reactions
- Serological methods
- Growth in different media
- Germ tube test
- Polymerase chain reaction

SABOURAUD'S DEXTROSE AGAR:

TEMPERATURE TOLERANCE-

The isolates are cultured on SDA and incubated at 45 °C ambient air for 72 hours and observed for growth. *C. albicans* species are temperature tolerant and show good growth at 45˚C incubation[34].

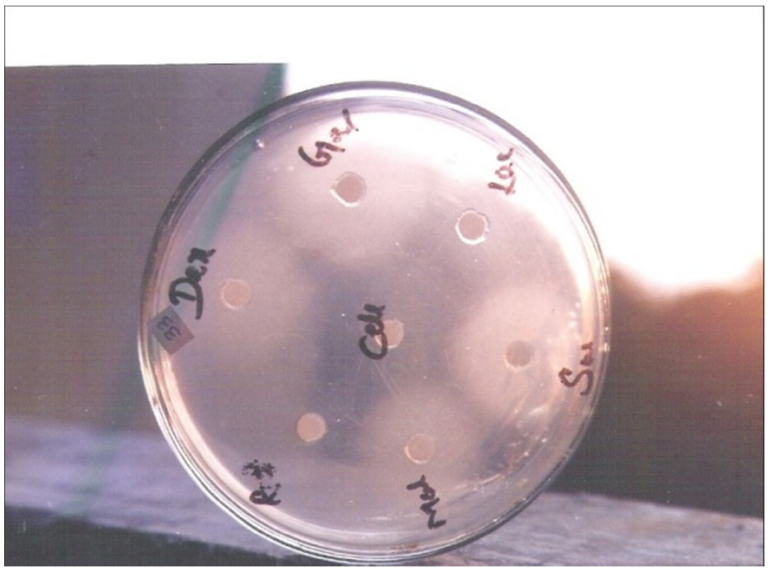
ADDITION OF TRIPHENYL TETRAZOLIUM CHLORIDE (PAGANO LEVIN MEDIA) –

C. albicans gives pale coloured colonies while other species produce different shades of pink[29].

BIOCHEMICAL REACTIONS:

CARBOHYDRATE ASSIMILATION TEST:

This method is used to determine assimilation of glucose, maltose, sucrose, lactose, raffinose, trehalose, galactose, Cellobiose, melibiose, and inositol by Candida yeasts. The sample is incubated at room temperature for about 24 hrs to deplete the carbohydrate reserve so that the sugar supplemented will be utilized properly and this rule out false negative results[54]. A suspension of yeast is flooded on the surface of a petri dish containing yeast nitrogen base and 0.4% bromocresol purple. Carbohydrate disks 6 or 13 mm in diameter are spaced evenly on the agar surface. The plates are incubated at 30°C for 24 to 72 h and examined for a colour change from purple to yellow or for halo of growth around the disks. Glucose is used as positive control, since all the species of Candida assimilate this carbohydrate[54, 55].



STRAIN	GLU	MAL	SUC	LAC	MEL	RAF	CEL	TRE	XYL
C. albicans	+	+	+	-	-	-	-	+	+
C.tropicalis	+	+	+	-	-	-	+	+	+
C. glabrata	+	+	-	-	-	-	-	+	-
C.parapsilosis	+	+	+	-	-	-	-	+	+
C. dubliensis	+	+	+	-	-	-	-	+	+
C.krusei	+	-	-	-	-	-	-	-	-

SUGAR FERMENTATION TEST:

Candida yeasts produce carbon dioxide and alcohol. Production of gas rather than a pH shift is indicative of fermentation. Fermentation of glucose, maltose, sucrose, and lactose is determined by inoculating carbohydrate fermentation tubes with 5 drops (0.2 ml) of the yeast-saline suspension. The tubes are incubated at 37°C and read after 24 and 48 h and again after 10 days for the presence of gas in the inverted Durham tubes. C. albicans ferments glucose and maltose but not sucrose and lactose.

Commercial systems using biochemical reactions:

- API 20C AUX system
- ID 32 C system
- RapID Yeast Plus system
- VITEK YBC system[52]

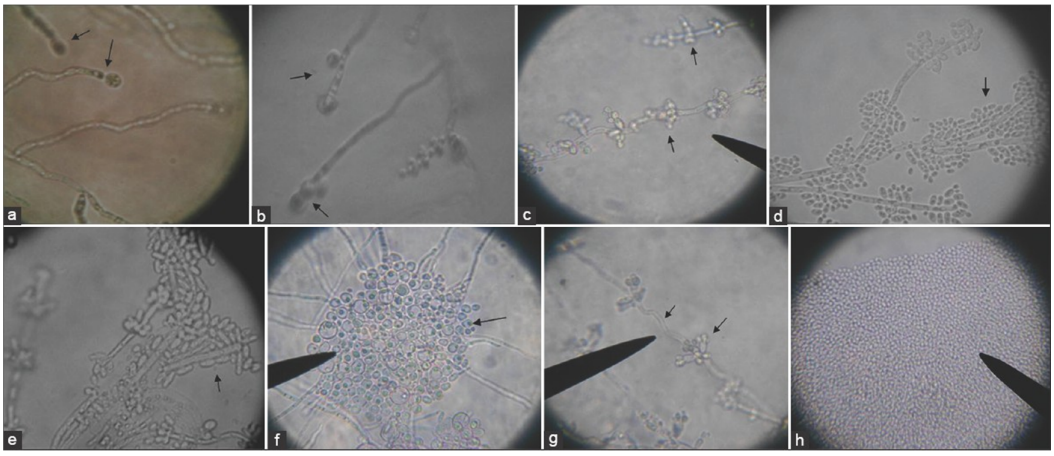
SEROLOGICAL METHODS:

Monospecific agglutinins can be used for identifying the members of the genus Candida by slide agglutination test. Separate sera are used for the specific identification of Candida guilliermondii, Candida krusei, Candida parapsilosis, and Candida pseudotropicalis. But Candida albicans cannot be antigenically delineated from Candida tropicalis. Candida positive cultures are subcultured on Sabouraud dextrose agar. 0.05 ml each of the factor sera and control physiological saline are added to the circles on the slides and 0.05 ml of a yeast suspension is then added to each of them. The reactants are mixed on a

platform rotary shaker and the agglutination reaction with each factor serum is recorded and compared with existing profiles[53].

CORN MEAL AGAR:

Chlamydospore production on Corn Meal Agar (CMA) is helpful for the identification of *Candida albicans***.** 17 g of Corn Meal Agar (CMA) incorporated with tween 80 is prepared. The colony from SDA medium is inoculated on CMA plates by slide culture technique. It is covered with a sterile cover slip and incubated at 25 ℃ for 72 hours. The specimen is stained with lactophenol cotton blue and Chlamydospore production is identified[34].



a) C. albicans showing large, thick-walled, terminal chlamydospores.
b) C. dubliniensis showing thick-walled, terminal chlamydospores in small bunches and pairs.
c) C. tropicalis showing blastoconidia in small groups along the pseudohyphae.
d) C. krusei showing cross matchstick- or tree-like appearance.
e) C. kefyr showing logs in stream appearance.

f) C. guilliermondii showing clusters of yeast cells with pseudohyphae with small groups of blastoconidia.

g) C. parapsilosis showing curved pseudophyphae with few blastoconidia.

h) C. glabrata showing oval budding yeast cells without pseudophyphae[34].

CHROMOGENIC AGAR:

It is used for identification of different Candida species based on the differential release of chromogenic breakdown products from various substrates following differential exoenzyme activity.

It is composed of,

Ingredients	**gms / Litre**
Peptone, special	15.000
Yeast extract	4.000
Dipotassium hydrogen phosphate	1.000
Chromogenic mixture	7.220
Chloramphenicol	0.500
Agar	15.000
Final pH (at 25°C)	6.3±0.2

Peptone special and yeast extract provides essential growth nutrients. Phosphate buffers the medium. Chloramphenicol suppresses the accompanying bacterial flora[33].

A single colony from a pure culture in SDA is inoculated into CHROMagar media and incubated at 35 ℃ for 48 hours after which colour changes are noted. Mixed yeast cultures in specimens can also be detected. The plates are read against a white background.

- *C. albicans* - light to medium green
- *C. dubliniensis* - dark green
- *C. tropicalis* - dark blue to metallic-blue
- *C.glabrata* - cream to white smooth colonies.
- *C. krusei* - light mauve to rose pink, flat colonies with a whitish border

C. glabrata and *C. parapsilosis* cannot be differentiated[31].

Chrome agar plate showing different Candida species. (a) Candida albicans, (b) C. dubliniensis, (c) C. tropicalis, (d) C. glabrata and (e) C. krusei.

NICKERSON'S MEDIA:

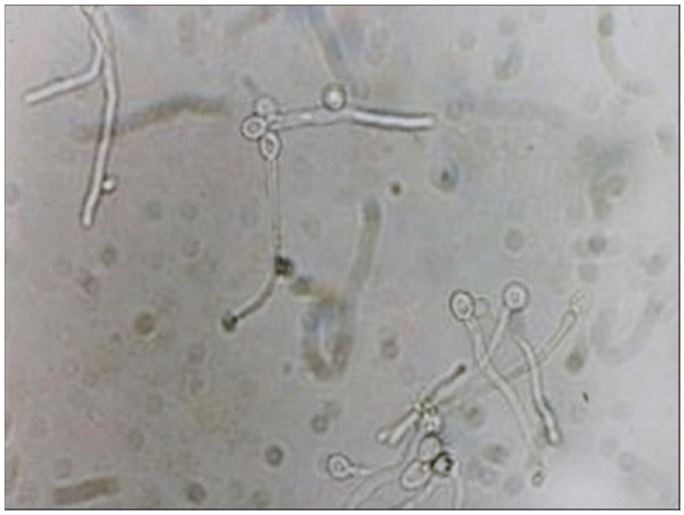
It is a differential medium of complex bismuth salts and gives light and dark coloured colonies. But it does not adequately differentiate between Candida species or from bacteria as most micro organisms produce black colour[29].

PHOSPHOMOLYBDATE AGAR:

C. albicans appear blue while others are green. But it gives high false positive and false negative results[29].

GERM TUBE TEST:

Germ tube formation was first reported by Reynold and Braude in 1956 and hence, the germ tube test is also known as a "Reynolds- Braude Phenomenon". The daughter cells arising from the round mother cell without constriction at their origin are referred to as germ tubes. It is a rapid test based ability of *C. albicans* to produce germ tubes when incubated in pooled human or horse serum. The colony from culture in Sabouraud's dextrose agar is inoculated in 0.5 ml serum and incubated at 37˚C for 2 to 3 hours. Subsequently the sample is examined for germ tube production for up to 5 minutes by using a light microscope under high power. A minimum of five germ tubes should be present in entire wet mount preparation for diagnosis[32].



POLYMERASE CHAIN REACTION:

Species-specific primers are used for detection of PCR amplified ribosomal DNAs (rDNAs) of commonly encountered *Candida* species like *C. albicans, C. parapsilosis, C. tropicalis* and *C. glabrata* by Southern hybridization. Different types of PCR used for speciation of Candida are,

- Multiplex PCRs
- snPCR or seminested PCR - 100 times more sensitive than the multiplex PCRs[49].
- PCR-EIA identification matrix[50].
- RAPD-PCR - random amplification polymorphism DNA PCR

Species-specific probes are also designed to discriminate between two species that have phenotypic characteristics in common like *C. dubliniensis* and *C. albicans.*

ANTIFUNGAL SUSCEPTIBILITY TESTING:

The majority of cases of vulvovaginal candidiasis are caused by *Candida albicans*; however, episodes due to non-albicans species of *Candida* appear to be increasing. Most non-albicans *Candida* species have higher azole MICs and infections they cause are often difficult to treat. MIC or minimum inhibitory concentration is the lowest concentration of drug capable of preventing microbial growth.

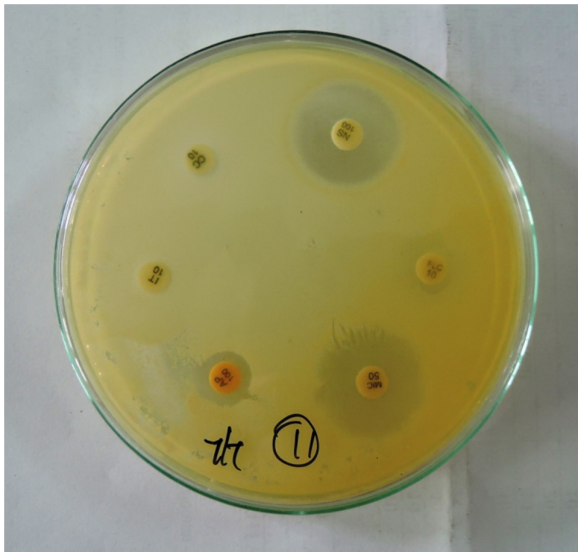
Resistance to antifungal drugs occur due to:

- Alterations in sterol biosynthesis
- Alteration in the uptake of drugs
- Bypass
- Alteration or overproduction of target enzymes 14 α demethylase, which lowers its affinity for fluconazole
- Increased expression of the ERG11 gene encoding 14 α demethylase
- Over expression of genes coding for membrane transport proteins of the ABC transporter (CDR1 / CDR2) or the major facilitator (MDR1) superfamilies
- Switching (the ability of Candida species to generate a variety of phenotypes) [46].

DISK DIFFUSION METHOD:

The antifungal susceptibility testing is done on Mueller Hinton Agar using the colonies directly from the Chromagar plates. Drug disks tested are -

Fluconazole, Clotrimazole, Miconazole, voriconazole, Itraconazole and Nystatin. The plates are incubated at 37ºC and examined after 24 hours of incubation. The zones of inhibition are measured in millimeter and the results are interpreted using validated CLSI (Clinical and laboratory standard institute) interpretive breakpoints.

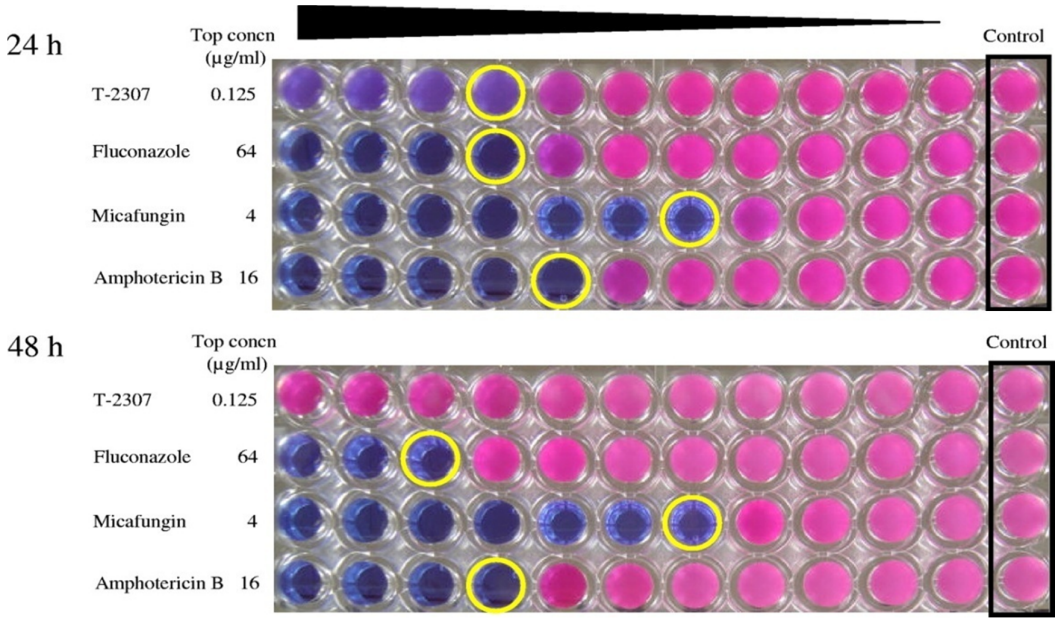


BROTH DILUTION METHOD:

Serial dilution of antifungal to be tested is done (0.125,0.25,0.5,1,2,4,8,16,32,64)ug/ml. Sterile microdilution plates (96-u-shaped wells) are used. Rows 1-10 contain the series of drug dilutions in 100 ul volumes starting with the concentration of 0.125 ug/ml. 100 ul of inoculums suspension are added to each well. The eleventh well is kept as control, 100 ul of inoculums suspension and 100 ul of drug free medium are added. The plates are covered, incubated at room temperature and examined after 48 and 72 hours of incubation[58].

MIC range (µg/mL)

Drug	Susceptible	Intermediately susceptible	Resistant
Fluconazole	≤ 8	16–32 (S-DDa)	>32
Itraconazole	≤ 0.125	0.25–0.5 (S-DDa)	>0.5



OTHER METHODS[60]:

- Flow cytometry - alterations in the fungal cell viability observed and the minimum fluorescence-enhancing concentration (MFEC) is assessed.
- MALDI – TOF Matrix-assisted laser desorption/ionization - time-of-flight mass spectrometer – 3 hours incubation in the presence of "breakpoint" concentrations of antifungals.
- Isothermal microcalorimetry - measures the thermal variations induced by the action of antifungals and estimates minimal heat inhibitory concentration (MHIC).

TREATMENT OF CANDIDIASIS:

RECOMMENDED REGIMENS FOR VAGINAL CANDIDIASIS[59]:

Intravaginal and oral therapy provides equally effective treatment for vaginal candidiasis.

AZOLE ANTIFUNGALS:

Mechanism of action: inhibition of CYP dependent enzyme lanosterol 14α demethylase with resultant inhibition of conversion of lanosterol to ergosterol. It is fungistatic.

Dosage and administration:

Oral preparations include

Fluconazole 150mg as a single dose

Itraconazole 200mg twice daily for one day

Intravaginal treatments include,

Butoconazole –

2% cream 5 g daily for 3 days or 2% sustained release cream 5 g for 1 day

Clotrimazole –

1% cream 5 g daily for 7 days or 2% cream 5 g daily for 3 days

100-mg vaginal tablet for 7 days or 200-mg vaginal tablet for 3 days

500mg vaginal tablet once

Miconazole -

2% cream 5 g daily for 7 days

100-mg vaginal suppository for 7 days

200-mg or 400 mg vaginal suppository for 3 days

1,200-mg vaginal suppository once

Nystatin - 100,000-unit vaginal tablet for 14 days

Tioconazole - 6.5% ointment 5 g once

Terconazole –

0.8% cream 5 g for 7 days or 0.8% cream 5 g for 3 days

80-mg vaginal suppository once

Econazole - vaginal pessary 150mg as a single dose

Only topical preparations should be used during pregnancy.

Uncomplicated VVC - Overall standard single dose treatments are as effective as longer courses.

C. glabrata – intrinsically resistant to fluconazole but higher doses (12mg/kg) can be given[62].

Complicated VVC or RVVC:

Repeat dose of fluconazole 150mg after 3 days or 10 to 14 days of topical therapy

Maintenance therapy –

- Clotrimazole 500mg vaginal suppository once weekly
- Oral ketoconazole 100mg once daily
- Oral fluconazole 150 mg once weekly
- Oral itraconazole 400mg once monthly or 100mg once daily

All maintenance regimes should be continued for 6 months.

Adverse effects:

- Nausea, vomiting, headache and abdominal pain
- Drug interactions
- Raised creatinine levels (fluconazole)
- Hypokalemia (itraconazole)
- Antiandrogenic effects (ketoconazole)
- Skin rash

NYSTATIN:

It gives a cure rate of 70 –90% for candida, but may be useful in women with an organism with reduced sensitivities to azole drugs.

Mechanism of action: Nystatin binds to ergosterol, a major component of the fungal cell membrane and forms pores in the membrane that lead to K^+ leakage, acidification, and death of the fungal cell.

Dosage and administration: The dose of a pessary is 100,000 units, 1 – 2 pessaries once at night for 14 days.

Adverse effects:

1. Itching and burning.
2. Hypersensitivity reactions, including Stevens-Johnson syndrome and acute generalized exanthematous pustulosis.

OTHER DRUGS:

- Vaginal boric acid - gelatine capsule at a dosage of 600 mg daily for 14 days.
- Amphotericin B - suppositories 50 mg nightly for 14 days.
- Flucytosine cream – 17% alone or in combination with 3% AmB cream administered daily for 14 days

PROBIOTICS:

28 days BD of probiotic L. rhamnosus GR-1 and L. reuteri RC-14

Mechanism of action:

- Lipoteichoic acid immune modulation via the small intestine
- Inhibition of the growth of C. albicans in the vagina
- Reduced ascension of yeast from the rectum to vagina

Adverse effect - high risk of bacteremia if invasive procedures are performed

FOLLOW UP:

- Follow up is required only in women with persistent or recurrent symptoms. All such women should have at least one speciated culture.

- Recurrent vulvovaginal candidiasis (four or more symptomatic episodes per year) - document frequency, establish diagnosis and confirm by culture and exclude risk factors (e.g. diabetes, underlying immunodeficiency, corticosteroid use, frequent antibiotic use)
- MIC testing should be performed in refractory infection
- Candida hypersentitivity should be ruled out
- Partner management can be tried for RVVC

VACCINES[12]

- Als-3 alum
- Virosomal Sap2
- b-glucan-CRM–conjugate/ MF59
- Beta-mannan- and Beta-mannoside conjugates
- HyR-1 (no adjuvant defined, probably alum)

Aims & Objectives

AIMS & OBJECTIVES

1. To study the prevalence of various candida species in female patients with vaginal discharge.

2. To study the susceptibility pattern of candida to commonly used antifungals

Materials and Methods

MATERIALS & METHODS

STUDY DESIGN

Prospective Observational study

STUDY GROUP

200 female patients attending the STD Out Patient Department, Institute of Venereology, Madras Medical College/RGGGH, Chennai are selected randomly. Both asymptomatic and symptomatic patients are taken for the study. Patients with complaints of vaginal discharge, vulval itching, lower abdominal pain, dyspareunia are taken as symptomatic patients.

The Institute ethics committee clearance was obtained and informed consent was taken from the women included in study group.

STUDY PERIOD

One year (August 2014 to July 2015)

INCLUSION CRITERIA

1. Patients aged >18 yrs to < 60 yrs.
2. Female patients attending STD OP with complaints of vaginal discharge, dyspareunia, dysuria and vulval itching.
3. Patients with nil complaints – Asymptomatic

EXCLUSION CRITERIA

1. Patients aged <18 yrs and >60 yrs.
2. Pregnant, lactating & menstruating women.

3. Patients who are not willing to participate in the study.
4. Those patients who had used antifungals and topical vaginal creams within 7 days prior to date of examination.

HISTORY

A detailed and thorough history was obtained pertaining to the following parameters:

- Age
- Occupation
- Socioeconomic status
- Marital and obstetric history
- Sexual history
- Contraceptive use
- Past, Personal, Treatment history
- History related to sexually transmitted infections as per the proforma enclosed.

GENITAL EXAMINATION

An external genital examination was done. Any growth, swelling, discharge was noted. Using clean and unlubricated Cusco's bivalve speculum, a thorough pelvic examination was done and any abnormalities in the vagina, cervix were noted. The amount, odour, colour and consistency of vaginal discharge were noted. Bimanual examination was done to note any adnexal tenderness.

SAMPLE COLLECTION

- The vaginal discharge was collected from the posterior fornix of the vagina or from vulva using a sterile cotton swab. Two swabs were used one for microscopic examination using Gram's stain and one for culture in Sabouraud's dextrose agar medium. A sample of discharge was collected using cover slip for wet mount examination.
- A cervical swab was taken for gonococcal culture.
- Blood samples were collected for VDRL and HIV antibody testing.

STUDY PRINCIPLE

Test 1 **:** Culture in Sabouraud's dextrose agar medium.

Test 2 **:** Subculture of growth if present in CHROM agar for speciation based on colour.

Test 3 **:** Germ tube test.

Test 4 **:** Antifungal susceptibility testing.

DIAGNOSIS

Candidiasis:

Diagnosis was made on the presence of budding yeast cells and pseudohyphae in Gram stain or KOH mount

Culture in sabouraud's dextrose agar - shiny, cream coloured, yeasty smelling and smooth surfaced colonies

Speciation with CHROM agar:

Green	–	C. albicans
Cream	–	C. glabrata
Blue	–	C. tropicalis
Pink	–	C. krusei

Germ tube test:

Positive	–	C. albicans

Antifungal susceptibility testing – disk diffusion method

Grams stain method:

- Vaginal swab was taken from the posterior fornix and smear was made, air dried and then heat fixed.
- Smear was stained with crystal violet solution for one minute and then washed under slow running water.
- The smear was again stained with Grams iodine solution for one minute and washed with slow running water.
- Next the smear was decolorized with acetone for 20-30 seconds and washed immediately in running water.
- The smear was counterstained with saffranin for 20-30 seconds and washed under slow flowing water.
- Smear was then air dried and viewed under microscope

STATISTICAL ANALYSIS

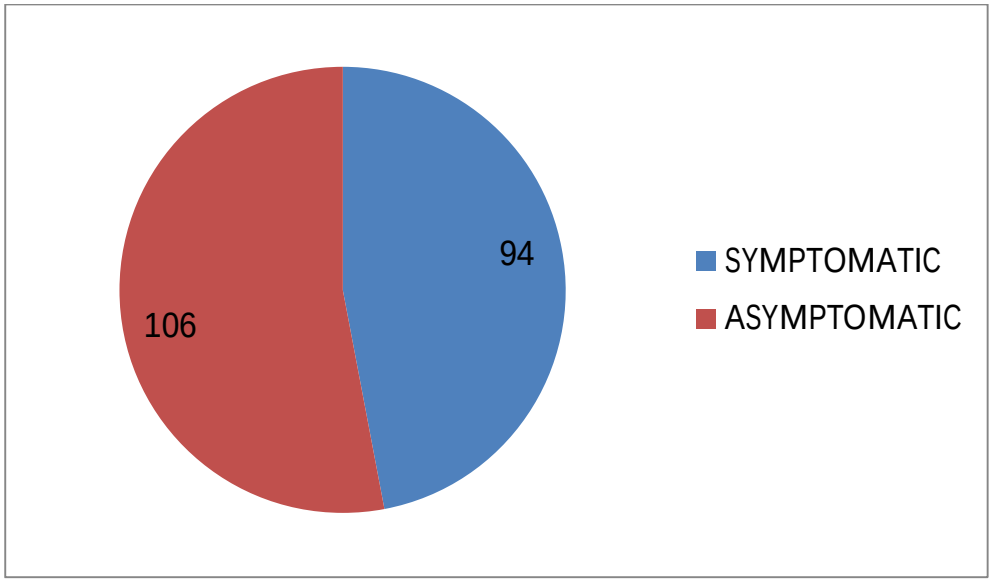
The data obtained was tabulated in Microsoft Excel Worksheet and computer based analysis was done. The prevalence of Candidiasis by microscopy and culture was noted. The prevalence of individual Candida species and antifungal susceptibility pattern was found out and statistical analysis of data done.

Observations & Results

OBSERVATION AND RESULTS

In this study we included a total of 200 female patients attending STD outpatient department. Out of these 106 were asymptomatic and 94 were symptomatic female patients.

Figure 1: TOTAL PATIENTS IN THE STUDY

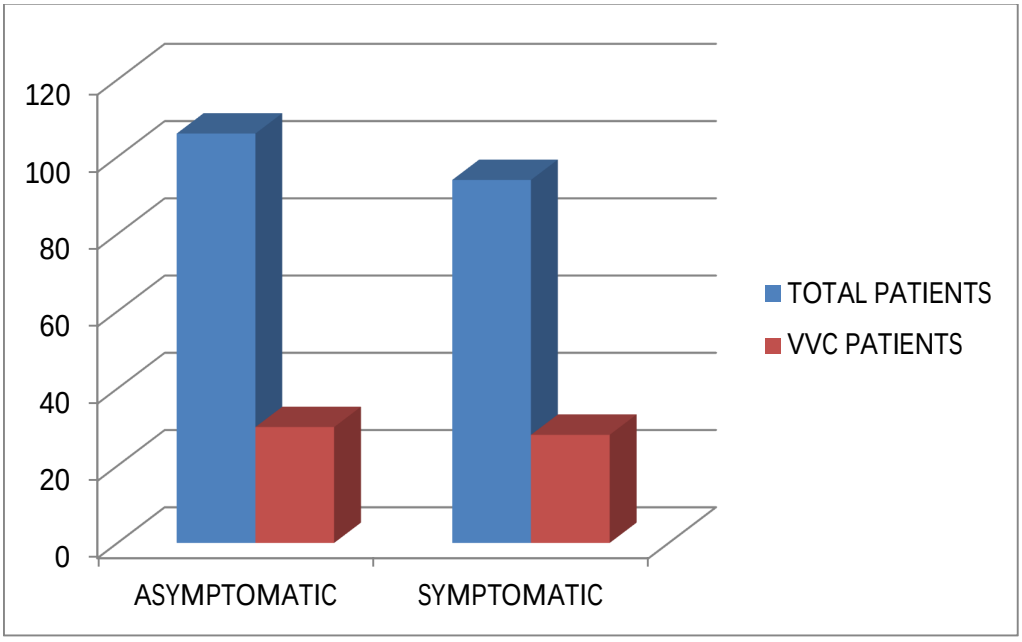


DISTRIBUTION OF VVC PATIENTS

Table 1: DISTRIBUTION OF VVC PATIENTS

DISTRIBUTION	TOTAL PATIENTS	VVC PATIENTS	PERCENTAGE (%)
ASYMPTOMATIC	106	30	28.3
SYMPTOMATIC	94	28	29.79

Figure 2: DISTRIBUTION OF VVC PATIENTS



AGE DISTRIBUTION

The age group of our study population ranged from 18 – 60 years. Of the 200 female participants in our study, 114 (57%) patients were in the age group of 26 – 40 years.

Table 2: AGE DISTRIBUTION

Age group	N = 200	PERCENTAGE (%)
18 - 25	21	10.5
26 - 30	40	20
31 - 35	34	17
36 - 40	40	20
41 - 45	19	9.5
46 - 50	21	10.5
51 - 55	12	6
56 - 60	13	6.5
Total	**200**	**100**

Figure 3: AGE DISTRIBUTION

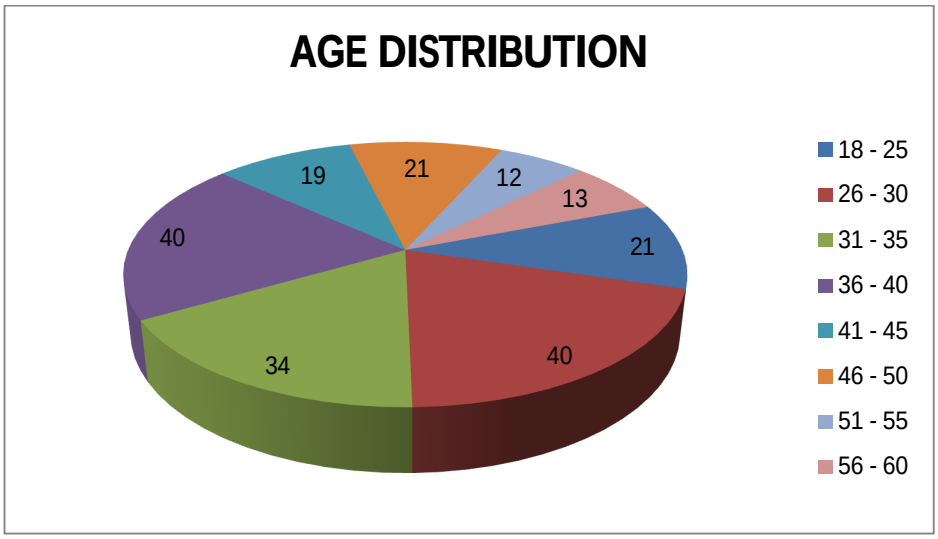
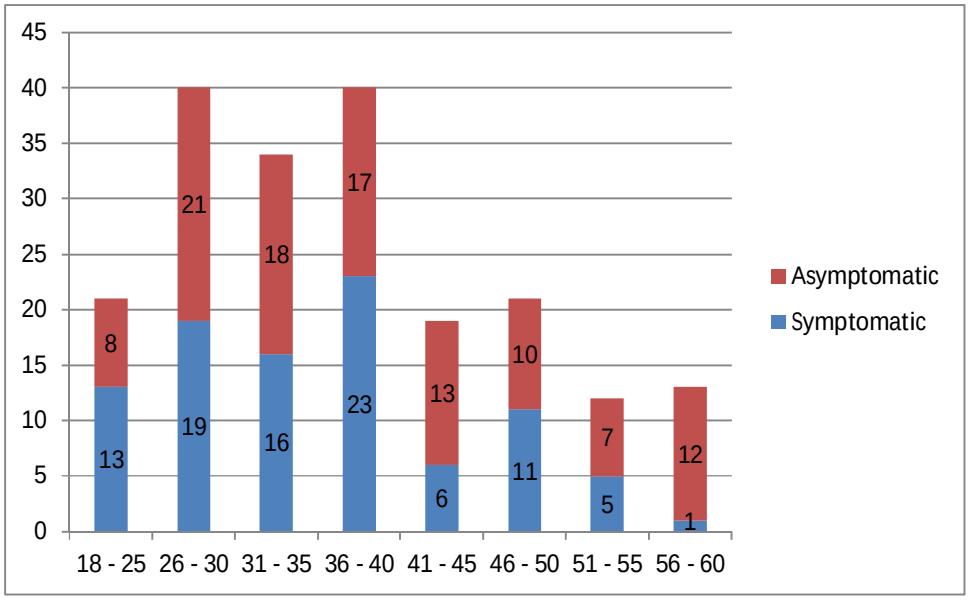


Figure 4: Age distribution of symptomatic and asymptomatic females

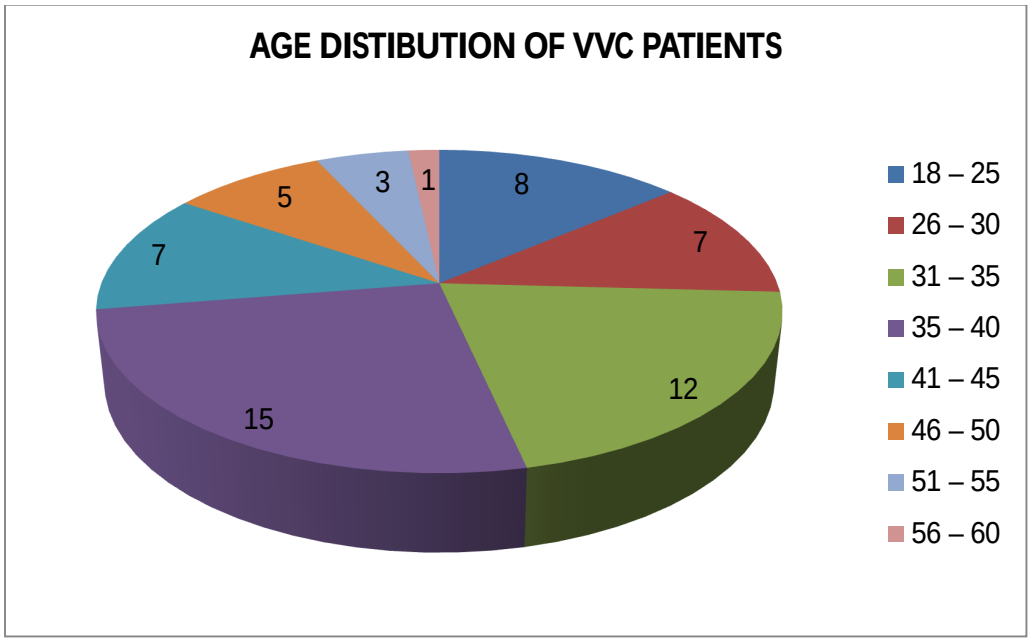


AGE DISTRIBUTION OF VVC PATIENTS

Table 3: DISTRIBUTION OF VVC PATIENTS BASED ON AGE

AGE	N = 200	VVC PATIENTS	PERCENTAGE (%)
18 – 25	21	8	4
26 – 30	40	7	3.5
31 – 35	34	12	6
36 – 40	40	15	7.5
41 – 45	19	7	3.5
46 – 50	21	5	2.5
51 – 55	12	3	1.5
56 – 60	13	1	0.5
TOTAL	**200**	**58**	**29**

Figure 5: **AGE DISTRIBUTION OF VVC PATIENTS**



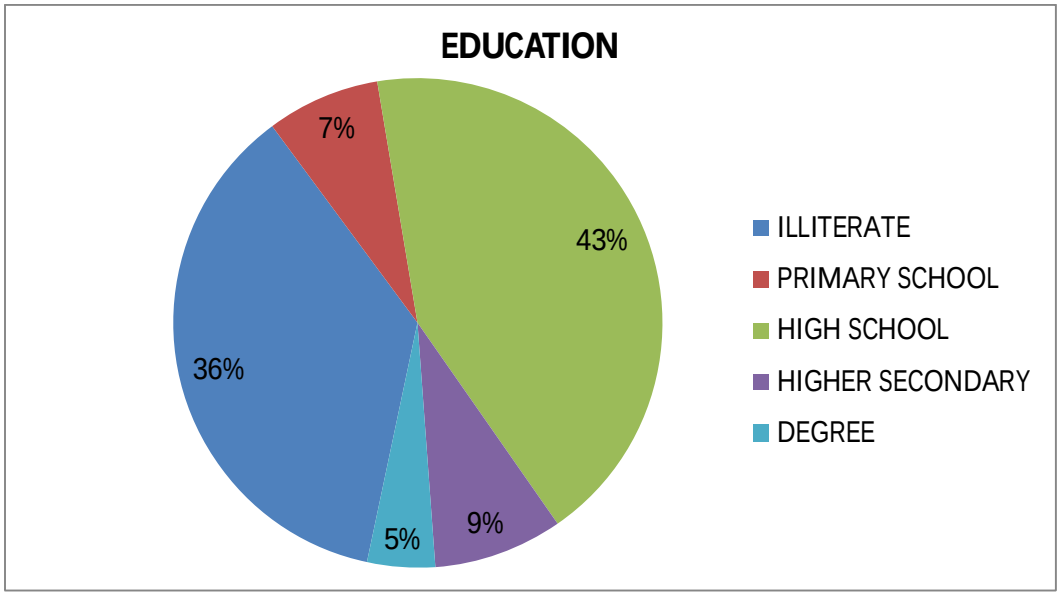
EDUCATIONAL STATUS

Majority (43%) of the females in the study population had completed high school.

Table 4: DISTRIBUTION BASED ON EDUCATION

EDUCATIONAL STATUS	TOTAL	PERCENTAGE (%)
ILLITERATE	73	36
PRIMARYSCHOOL	15	7
HIGH SCHOOL	86	43
HIGHER SECONDARY	17	9
DEGREE	9	5
TOTAL	**200**	**100**

Figure 6: DISTRIBUTION BASED ON EDUCATION



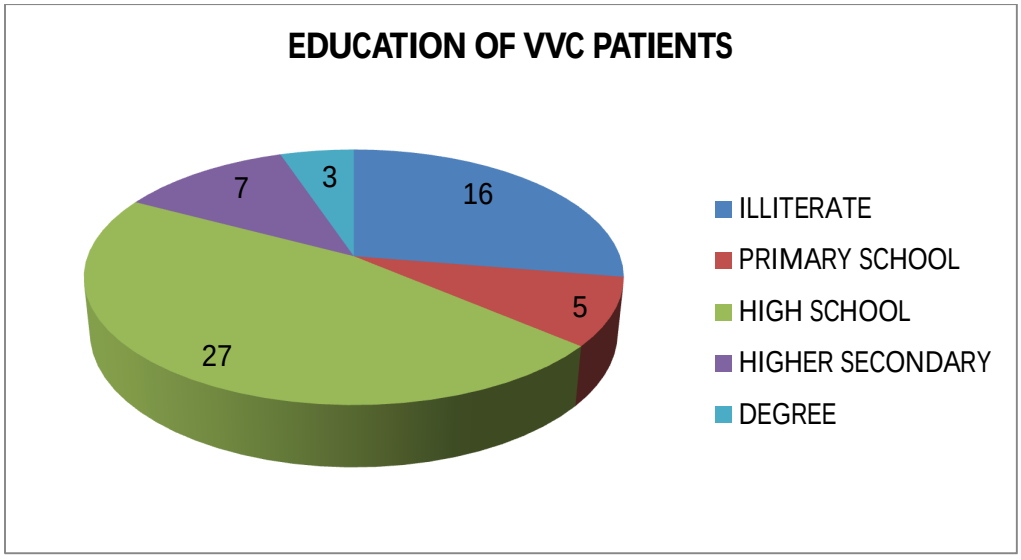
EDUCATION OF VVC PATIENTS

Prevalence of VVC was highest in those who had completed high school (13.5%).

Table 5: DISTRIBUTION OF VVC PATIENTS BASED ON EDUCATION

EDUCATION	N = 200	VVC PATIENTS (N = 58)	PERCENTAGE (%)
ILLITERATE	73	16	8
PRIMARY SCHOOL	15	5	2.5
HIGH SCHOOL	86	27	13.5
HIGHER SECONDARY	17	7	3.5
DEGREE	9	3	1.5
TOTAL	**200**	**58**	**29**

Figure 7: DISTRIBUTION OF VVC PATIENTS BASED ON EDUCATION



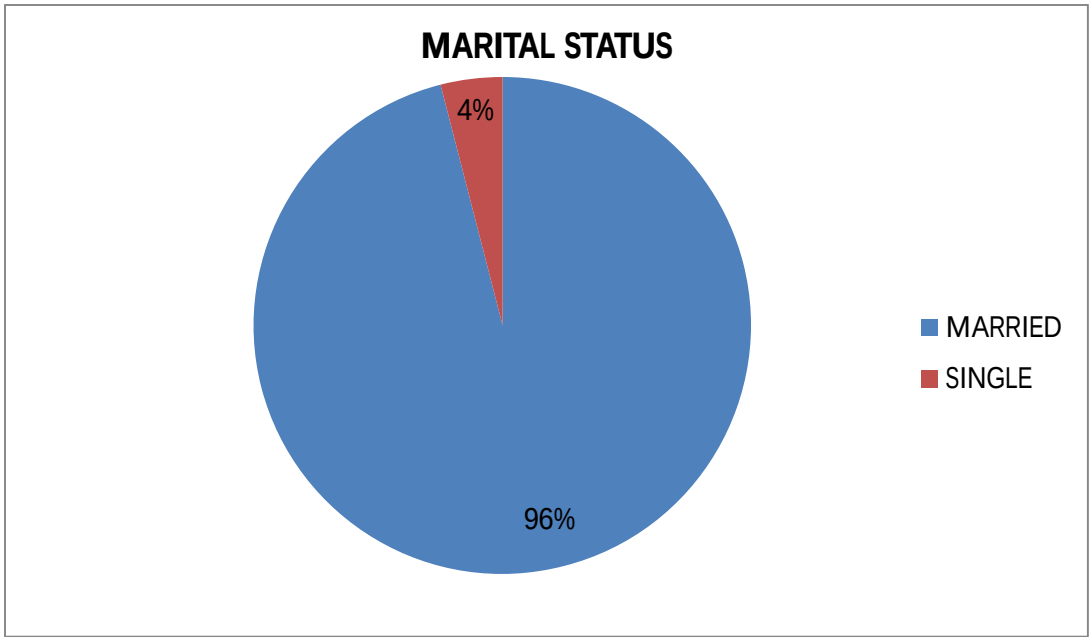
MARITAL STATUS

Of the 200 study population 192(96%) were married females and 8(4%) were unmarried females.

Table 6: MARITAL STATUS

MARITAL STATUS	N= 200	PERCENTAGE (%)
MARRIED	192	96
SINGLE	8	4

Figure 8: MARITAL STATUS



MARITAL STATUS OF VVC PATIENTS

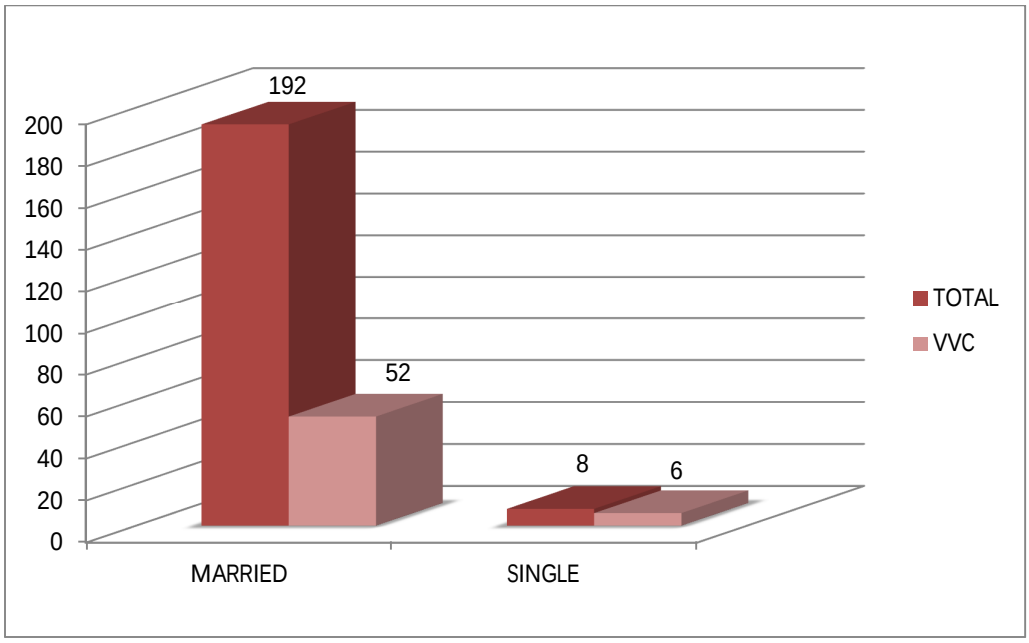
Out of the 192 married females 52 (27.08%) patients had VVC.

Out of the 8 unmarried females 6 (75%) patients had VVC.

Table 7: DISTRIBUTION OF VVC PATIENTS BASED ON MARITAL STATUS

MARITAL STATUS	N = 200	VVC	PERCENTAGE (%)
MARRIED	192	52	27.08
SINGLE	8	6	75

Figure 9: DISTRIBUTION OF VVC PATIENTS BASED ON MARITAL STATUS



DISTRIBUTION OF VVC IN SYMPTOMATIC AND ASYMPTOMATIC PATIENTS BASED ON MARITAL STATUS

75% (6) of the unmarried females were symptomatic and 25% (2) were asymptomatic.

Of the married females 45.83% (88) were symptomatic and 54.17% (104) were asymptomatic.

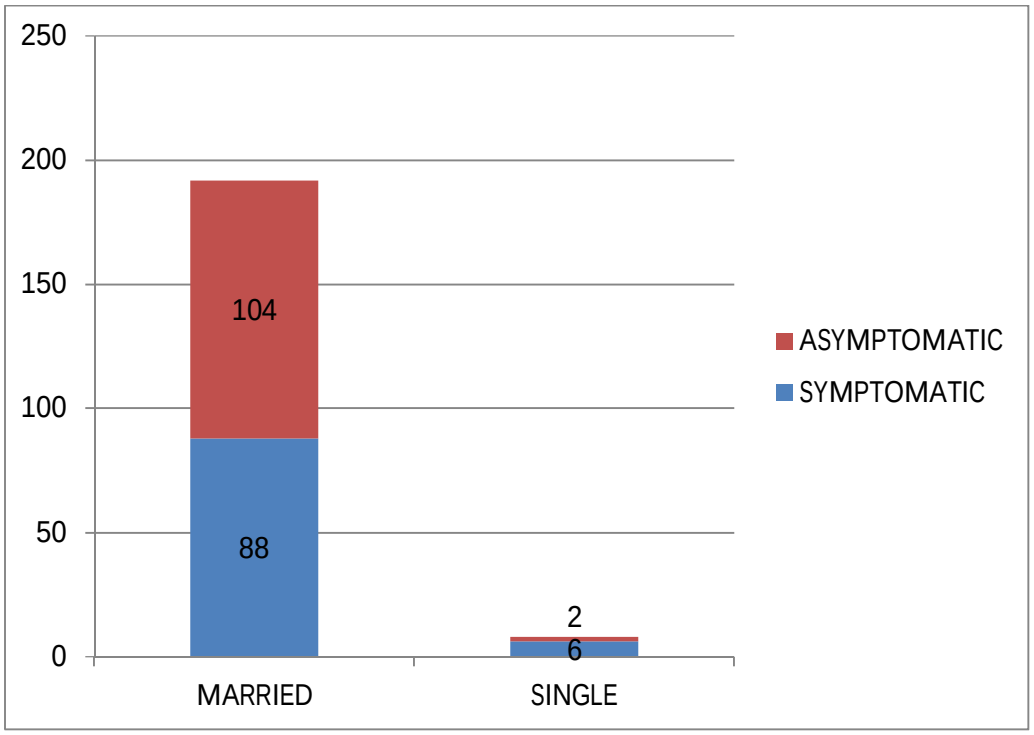
Table 8: DISTRIBUTION OF VVC IN SYMPTOMATIC FEMALES BASED ON MARITAL STATUS

MARITAL STATUS	N = 94	VVC IN SYMPTOMATIC	PERCENTAGE (%)
MARRIED	88	24	27.27
SINGLE	6	4	75

Table 9: DISTRIBUTION OF VVC IN ASYMPTOMATIC FEMALES BASED ON MARITAL STATUS

MARITAL STATUS	N= 106	VVC IN ASYMPTOMATICS	PERCENTAGE (%)
MARRIED	104	28	26.92
SINGLE	2	2	100

Figure 10: DISTRIBUTION OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS BASED ON MARITAL STATUS



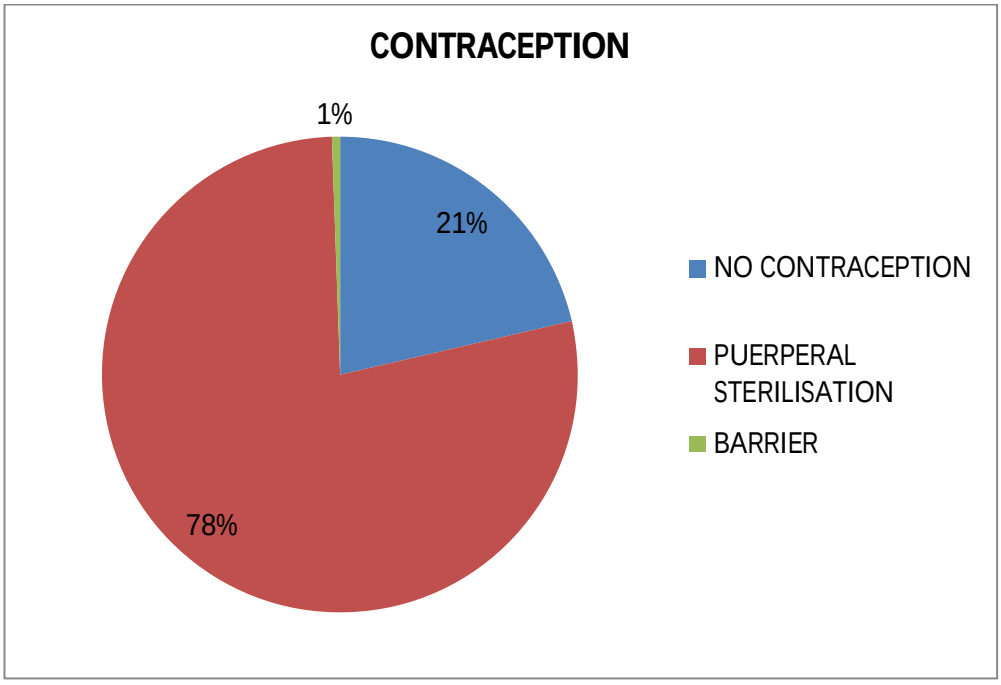
CONTRACEPTION

150 females (78.13%) of married women in our study group had undergone puerperal sterilisation.41 females (21%) followed no contraceptive method. Barrier method was followed by one female.

Table 10: DISTRIBUTION BASED ON CONTRACEPTIVE USE

CONTRACEPTION	N = 192	PERCENTAGE (%)
PUERPERAL STERILISATION	150	78.13
NO CONTRACEPTION	41	21.35
BARRIER	1	0.52

Figure 11: DISTRIBUTION BASED ON CONTRACEPTIVE USE



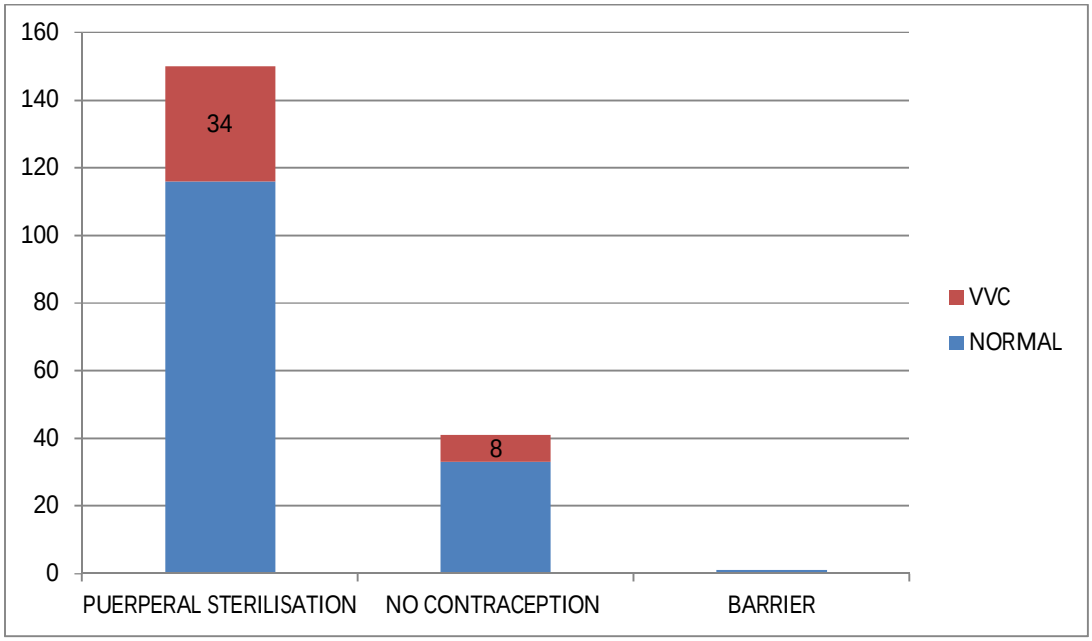
CONTRACEPTION AMONG VVC PATIENTS

22.67% patients who had undergone puerperal sterilisation had VVC when compared of 19.51% who practised no contraception.

Table 11: DISTRIBUTION OF VVC PATIENTS BASED ON CONTRACEPTION

CONTRACEPTION	N= 192	N = 52	PERCENTAGE (%)
PUERPERAL STERILISATION	150	34	22.67
NO CONTRACEPTION	41	8	19.51
BARRIER	1	0	0

Figure 12: DISTRIBUTION OF VVC PATIENTS BASED ON CONTRACEPTION



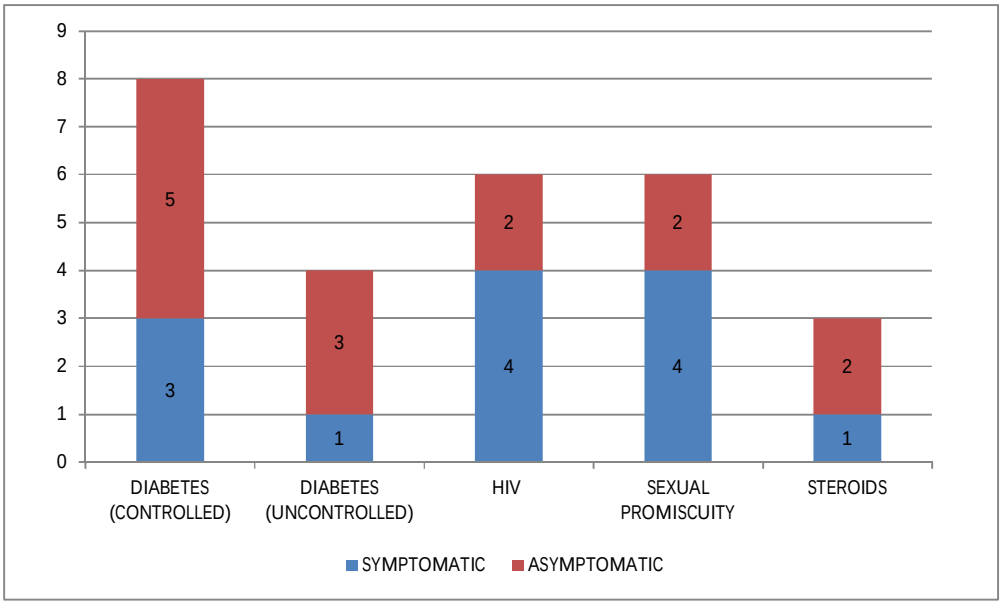
RISK FACTORS

The risk factors found in our study were controlled and uncontrolled diabetes, steroid intake, HIV and multiple sexual contacts.

Table 12: DISTRIBUTION OF PATIENTS BASED ON RISK FACTORS

RISK FACTORS	SYMPTOMATIC	ASYMPTOMATIC	PERCENTAGE (%)
NO RISK FACTORS	79	92	85.5
DIABETES	4	8	6
HIV	6	2	4
SEXUAL PROMISCUITY	4	2	3
STEROIDS	1	2	1.5
TOTAL	**94**	**106**	**100**

Figure 13: DISTRIBUTION OF PATIENTS BASED ON RISK FACTORS



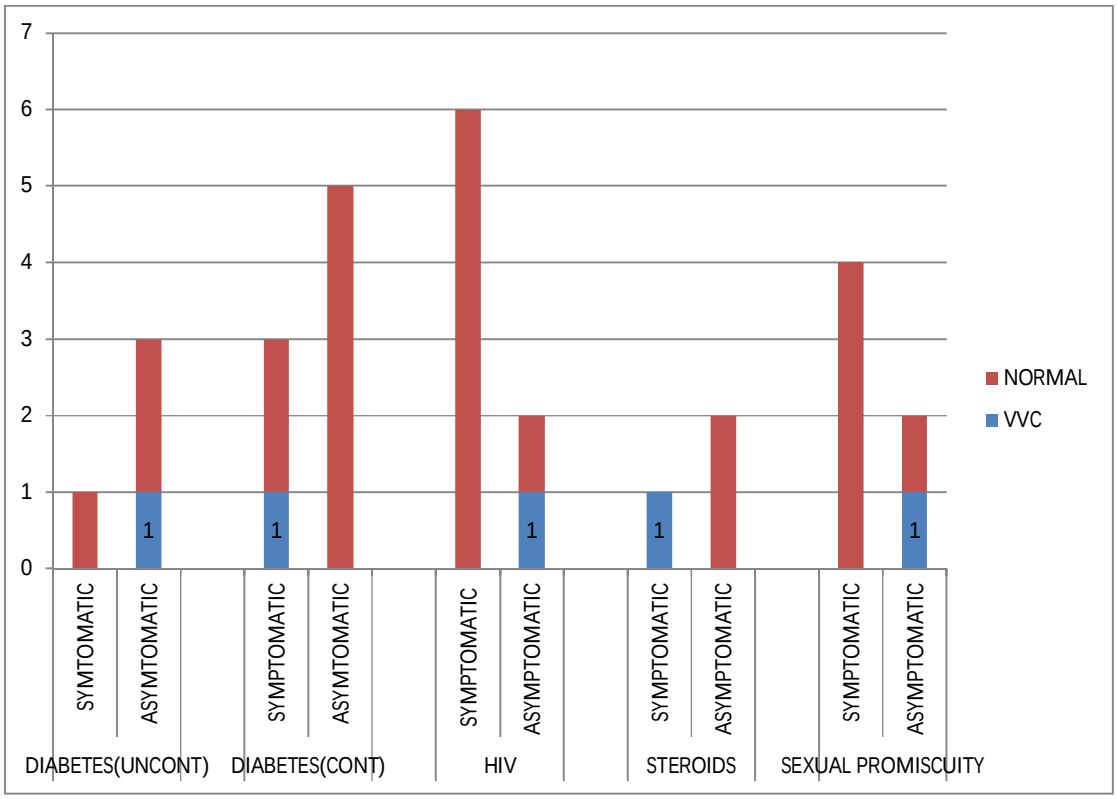
VVC IN PATIENTS WITH RISK FACTORS

Only 5 (17.24%) had VVC among females with known risk factors.

Table 13: DISTRIBUTION OF CANDIDIASIS BASED ON RISK FACTORS

RISK FACTORS	TOTAL N = 29	VVC N = 5	PREVALENCE PERCENTAGE (%)	PREVALENCE IN PATIENTS WITH NO RISK (%)
DIABETES	12	2	16.66	29.79
HIV	8	1	12.5	29.69
SEXUAL PROMISCUITY	6	1	16.66	29.38
STEROIDS	3	1	33.33	28.93

Figure 14: DISTRIBUTION OF CANDIDIASIS BASED ON RISK FACTORS



SEROLOGICAL STATUS

- **HIV STATUS**

  8 (4%) patients tested positive.

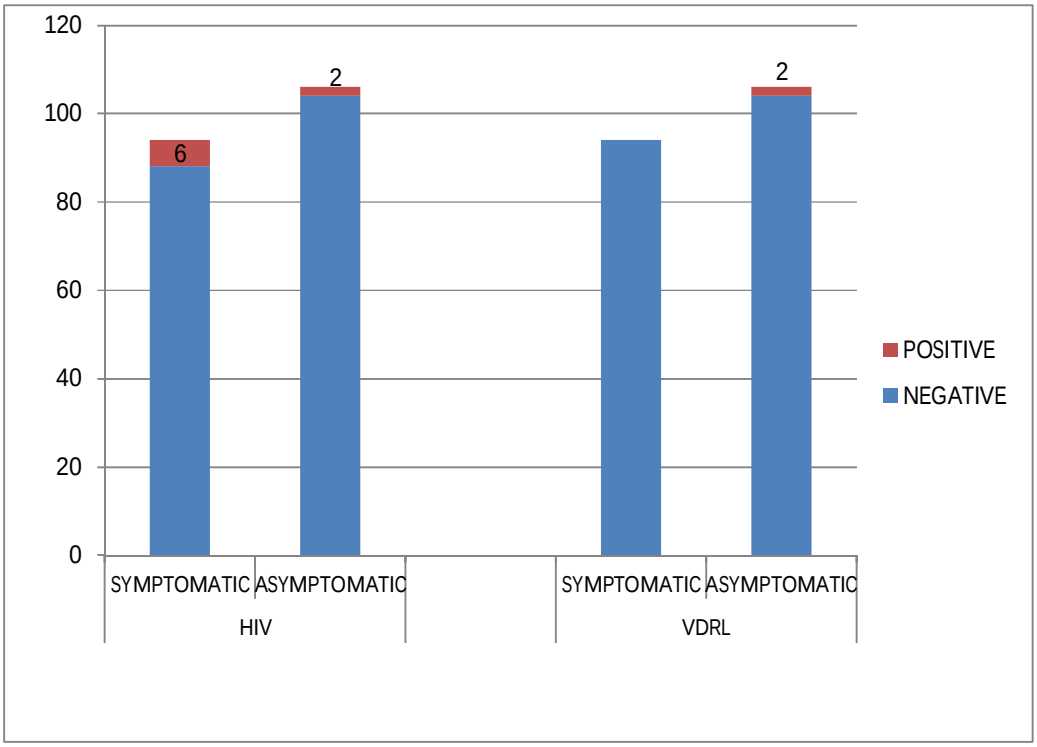
- **VDRL STATUS**

  Only 2 (1%) patients were VDRL reactive with one having 1:4 dilutions and the other patient having 1:1dilution. Both were TPHA positive.

Table 14: DISTRIBUTION BASED ON SEROLOGICAL STATUS

SEROLOGICAL STATUS	POSITIVES	PERCENTAGE (%)
HIV	8	4
VDRL	2	1

Figure 15: DISTRIBUTION BASED ON SEROLOGICAL STATUS



SEROLOGICAL STATUS OF VVC PATIENTS

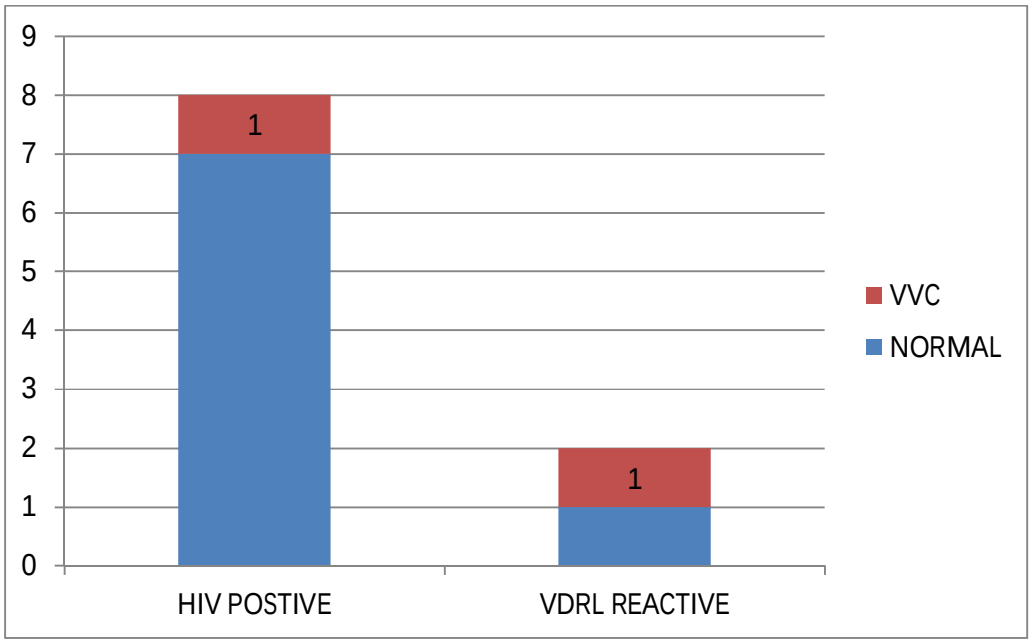
12.5% of HIV patients had Vulvovaginal Candidiasis.

50% of VDRL reactive patients had Vulvovaginal Candidiasis.

Table 15: DISTRIBUTION OF VVC PATIENTS BASED ON SEROLOGICAL STATUS

SEROLOGICAL STATUS	N = 200	VVC N = 58	PERCENTAGE (%)
HIV POSTIVE	8	1	12.5
VDRL REACTIVE	2	1	50
NORMAL	190	56	29.47

Figure 16: DISTRIBUTION OF VVC PATIENTS BASED ON SEROLOGICAL STATUS



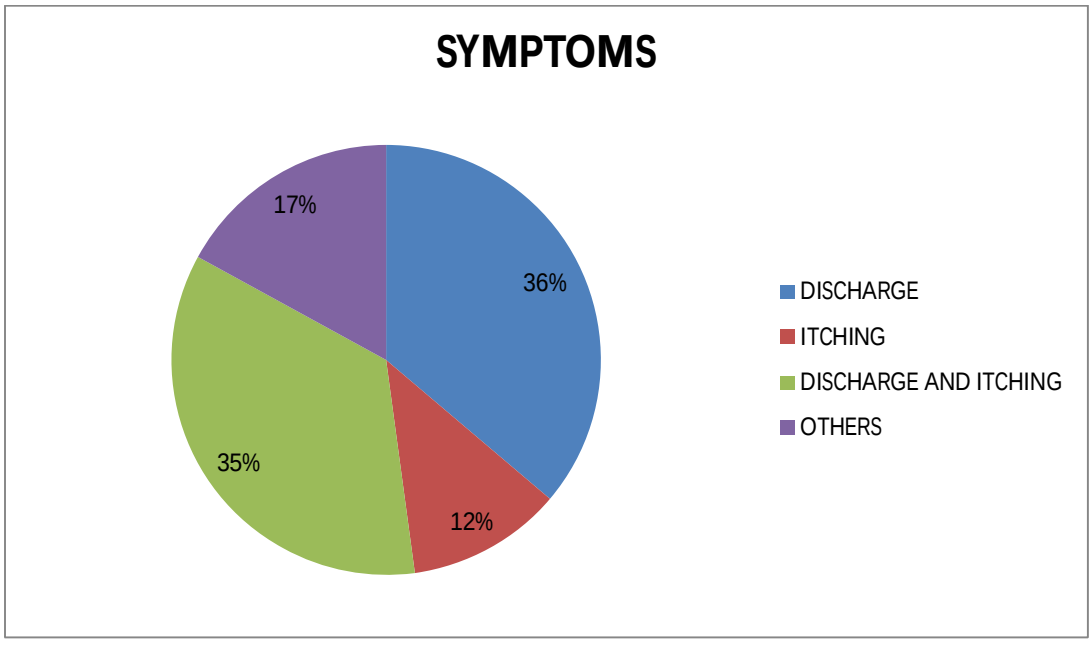
CLINICAL FEATURES

The most common complaint among symptomatic patients was vaginal discharge (36.17%).

Table 16: CLINICAL FEATURES

SYMPTOMS	N = 94	PERCENTAGE (%)
DISCHARGE	34	36.17
ITCHING	11	11.7
DISCHARGE AND ITCHING	33	35.11
OTHERS	16	17.02

Figure 17: DISTRIBUTION BASED ON SYMPTOMS



VVC BASED ON SYMPTOMS

Prevalence of VVC was highest (35.29%) in patients who complained of vaginal discharge.

Table 17: DISTRIBUTION OF VVC PATIENTS BASED ON SYMPTOMS

SYMPTOMS	TOTAL N = 94	VVC	PERCENTAGE (%)
DISCHARGE	34	12	35.29
ITCHING	11	3	27.27
DISCHARGE AND ITCHING	33	8	24.24
OTHERS	16	5	31.25

Figure 18: DISTRIBUTION OF VVC PATIENTS BASED ON SYMPTOMS

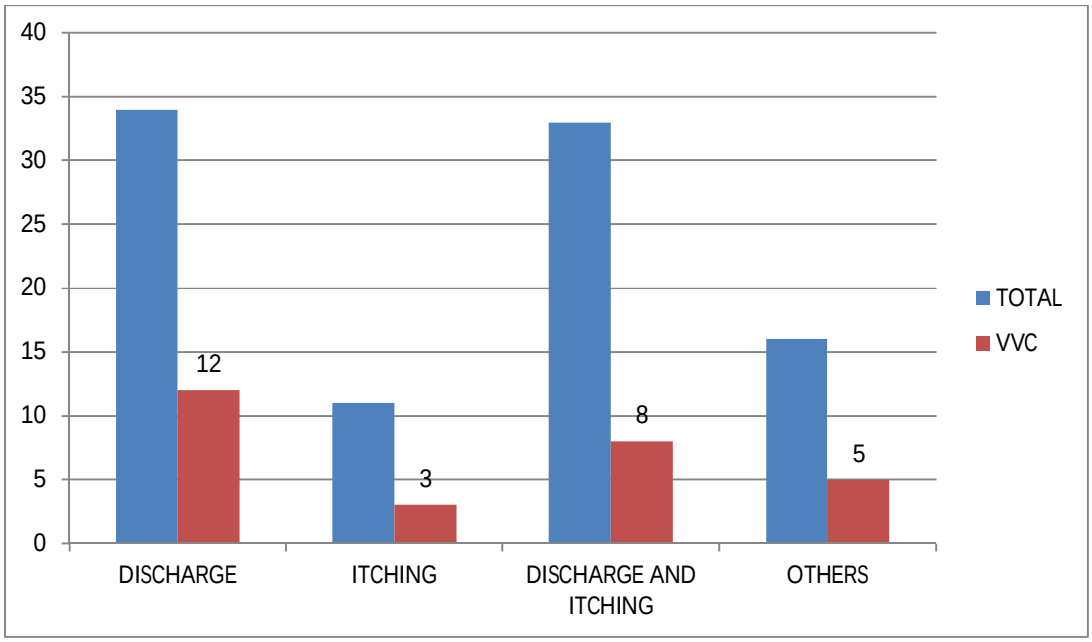
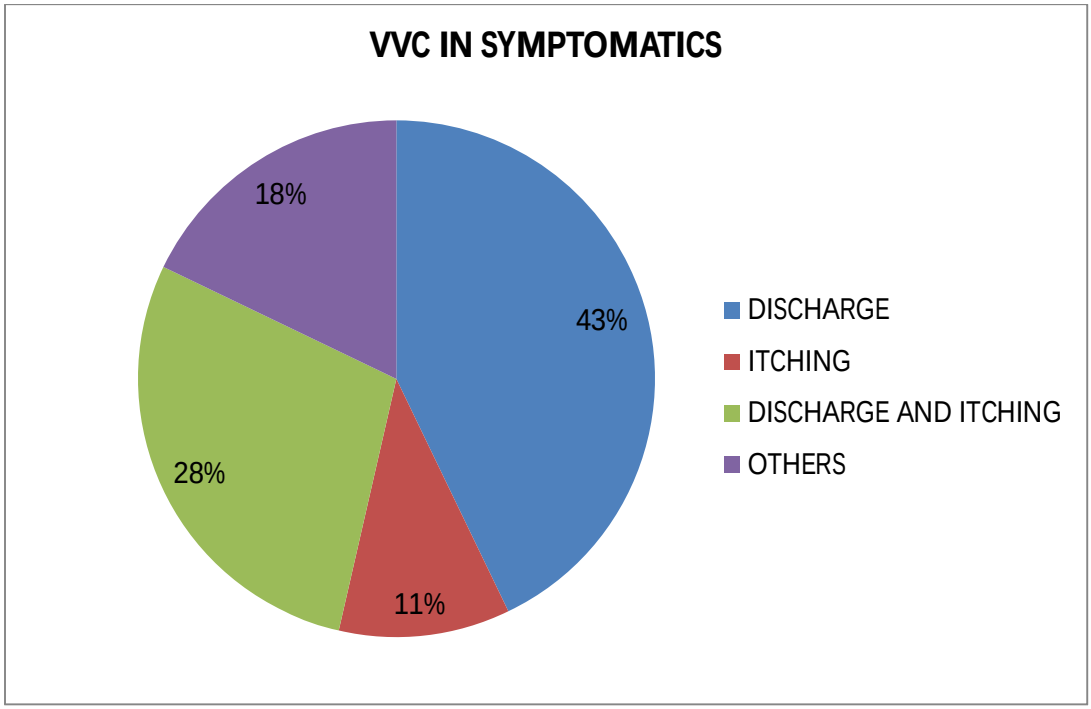


Figure 19: PERCENTAGE DISTRIBUTION OF VVC IN SYMPTOMATIC PATIENTS



Of the 28 VVC patients in symptomatic group, 43% were patients complaining of vaginal discharge, 11% were patients with itching, 28% were patients with both discharge and itching and 18% were patients with other symptoms like soreness and dyspareunia.

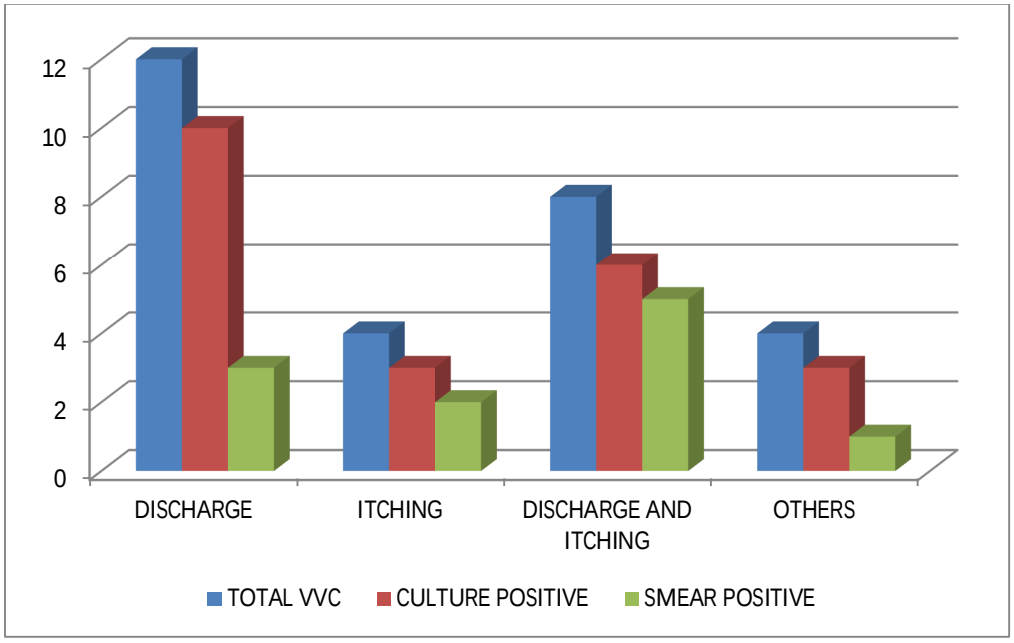
CULTURE AND MICROSCOPY IN SYMPTOMATIC VVC PATIENTS

Among 28 symptomatic VVC patients, 78.57% were culture positive while only 39.29% were smear positive.

Table 18: **CULTURE AND MICROSCOPY IN SYMPTOMATIC VVC PATIENTS**

SYMPTOMS	VVC	CULTURE PROVEN VVC	PERCENTAGE (%)	SMEAR POSITIVE VVC	PERCENTAGE (%)
DISCHARGE	12	10	83.33	3	25
ITCHING	4	3	75	2	50
DISCHARGE AND ITCHING	8	6	75	5	62.5
OTHERS	4	3	75	1	25
TOTAL	**28**	**22**	**78.57**	**11**	**39.29**

Figure 20: CULTURE AND MICROSCOPY IN SYMPTOMATIC VVC PATIENTS



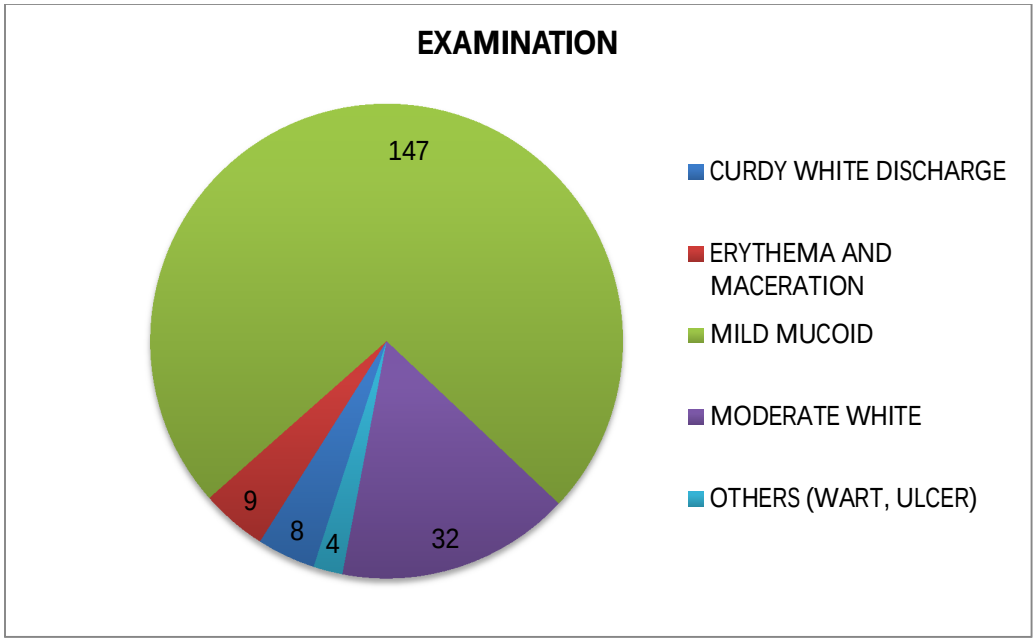
CLINICAL EXAMINATION

The most common finding on clinical examination of patients was mild mucoid discharge (73.5%).

Table 19: DISTRIBUTION BASED ON CLINICAL FINDINGS

CLINICAL FINDINGS	N = 200	PERCENTAGE (%)
MILD MUCOID	147	73.5
MODERATE WHITE	32	16
ERYTHEMA AND MACERATION	9	4.5
CURDY WHITE DISCHARGE	8	4
OTHERS (WART, ULCER)	4	2
TOTAL	**200**	**100**

Figure 21: DISTRIBUTION BASED ON CLINICAL FINDINGS

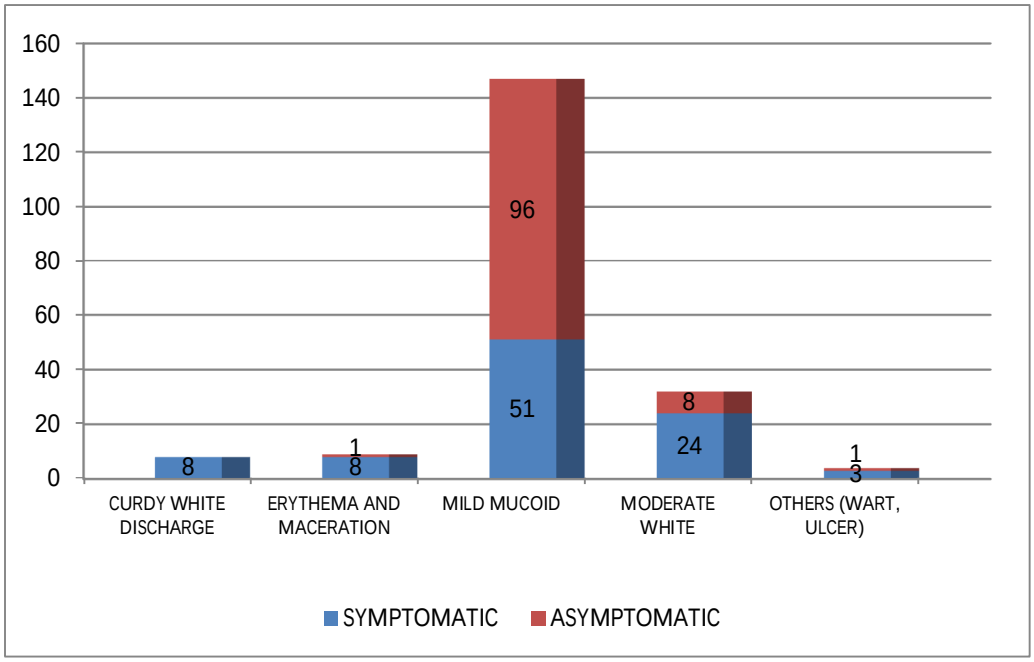


CLINICAL FINDINGS OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS

Table 20: DISTRIBUTION OF SYMPTOMATIC AND ASYMPTOMATIC PATIENTS BASED ON CLINICAL FINDINGS

EXAMINATION	TOTAL	SYMPTOMATIC	PERCENTAGE	ASYMPTOMATIC	PERCENTAGE
CURDY WHITE	8	8	100	0	0
MACERATION	9	8	88.9	1	11.11
MILD MUCOID	147	51	34.7	96	65.3
MODERATE WHITE	32	24	75	8	25
OTHERS	4	3	75	1	25

Figure 22: DISTRIBUTION OF SYMPTOMATIC AND ASYMPTOMATIC FEMALES BASED ON CLINICAL EXAMINATION



CLINICAL EXAMINATION IN VVC PATIENTS

Maximum number (40) of VVC cases are seen in patients with mild mucoid discharge. 75% of those with curdy white discharge had Vulvovaginal Candidiasis.

Table 21: DISTRIBUTION OF VVC PATIENTS BASED ON CLINICAL EXAMINATION

CLINICAL FINDINGS	N = 200	VVC	PERCENTAGE
MILD MUCOID	147	40	27.21
MODERATE WHITE	32	7	21.88
CURDY WHITE	8	6	75
MACERATION	9	4	44.44
OTHERS	4	1	25
TOTAL	**200**	**58**	**29**

Figure 23: DISTRIBUTION OF VVC PATIENTS BASED ON CLINICAL EXAMINATION

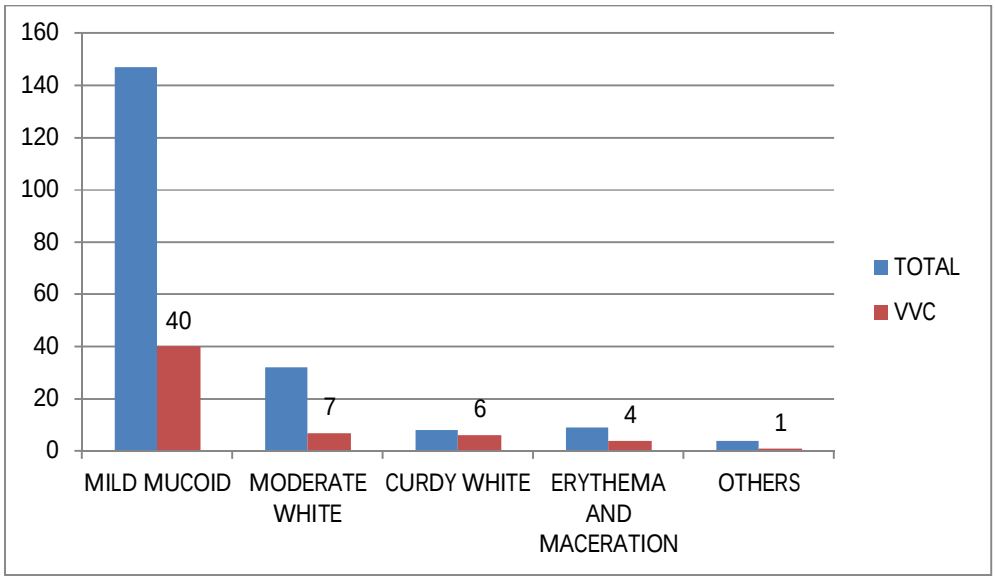
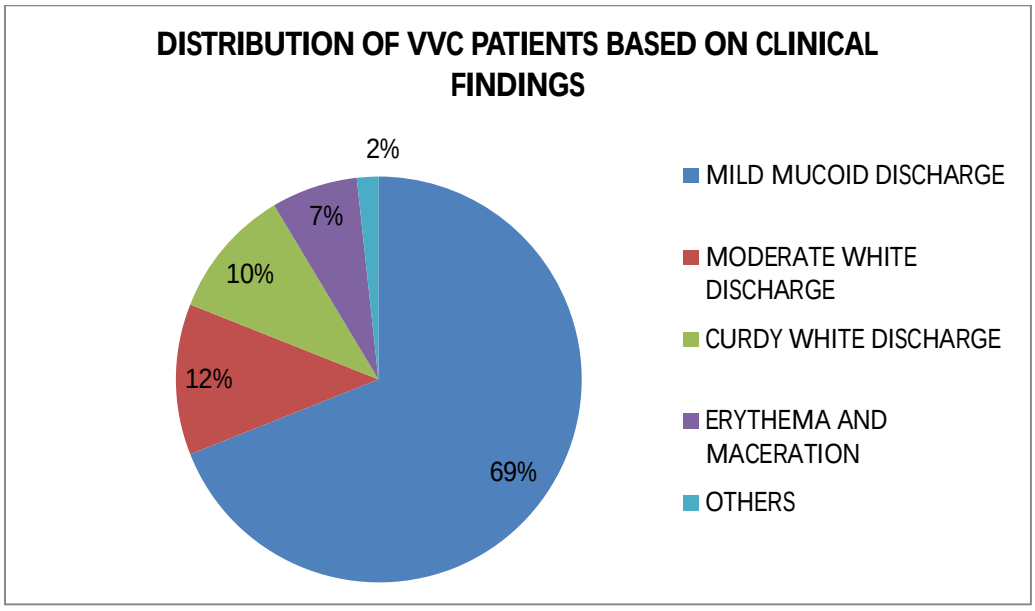


Figure 24: **PERCENTAGE DISTRIBUTION OF VVC PATIENTS BASED ON CLINICAL FINDINGS**



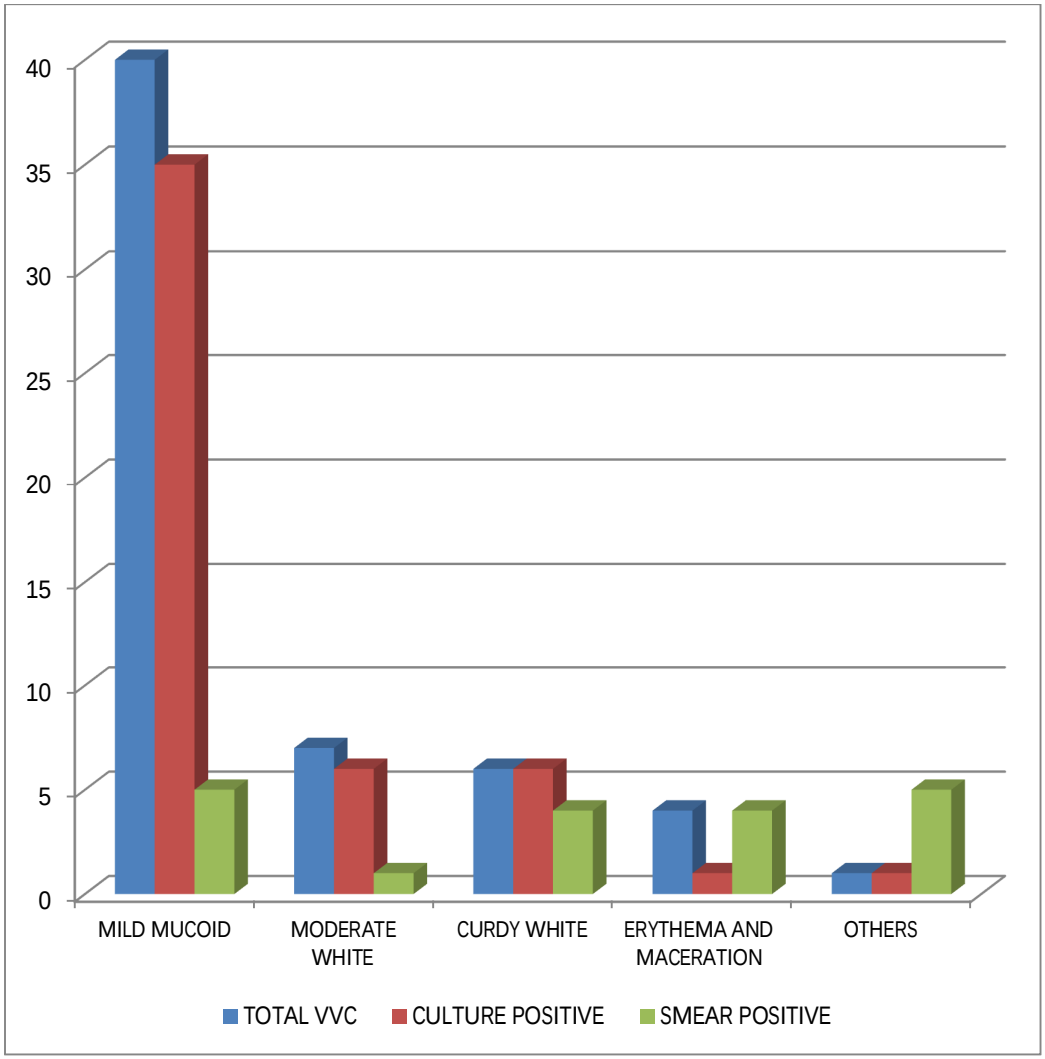
Among the 58 patients with VVC, 40 (69%) had mild mucoid discharge, 7 (12%) had moderate amount of white discharge, 4 (7%) had erythema and maceration of vulva, 6 (10%) had curdy white discharge and 1 (2%) patient had other findings like wart, erosion and vesicles.

CULTURE AND MICROSCOPY IN VVC PATIENTS BASED ON CLINICAL EXAMINATION

Table 22: CULTURE AND MICROSCOPY IN VVC PATIENTS BASED ON CLINICAL EXAMINATION

CLINICAL EXAMINATION	TOTAL VVC	CULTURE POSITIVE	PERCENTAGE (%)	SMEAR POSITIVE	PERCENTAGE (%)
MILD MUCOID	40	35	87.5	5	12.5
MODERATE WHITE	7	6	85.71	1	14.29
CURDY WHITE	6	6	100	4	75
ERYTHEMA AND MACERATION	4	1	25	4	100
OTHERS	1	1	100	0	0
TOTAL	**58**	**49**	**84.48**	**14**	**24.14**

Figure 25: CULTURE AND MICROSCOPY IN VVC PATIENTS BASED ON CLINICAL EXAMINATION



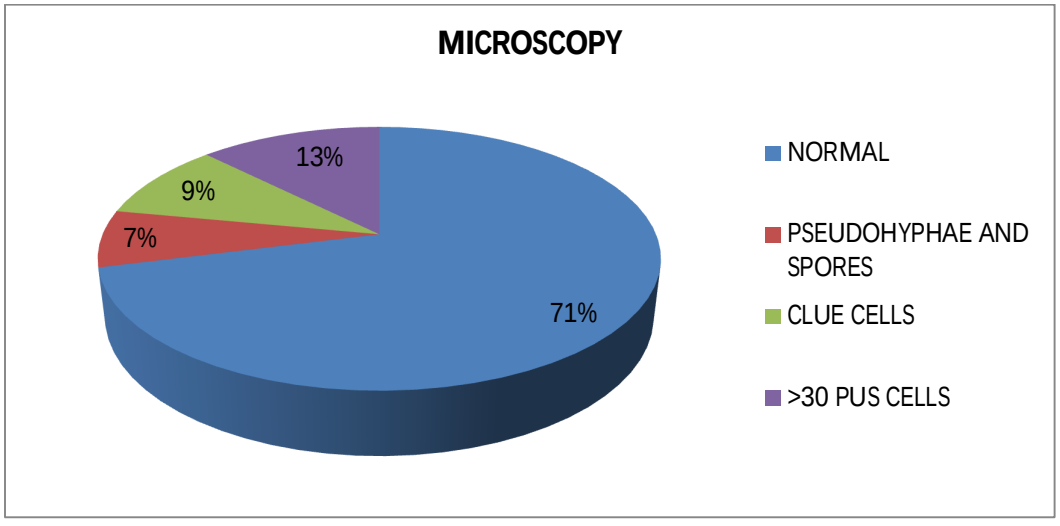
MICROSCOPY

Microscopic examination of vaginal discharge was normal in 142 (71%) females. 14 (7%) females had pseudohyphae and spores on microscopic examination. 19 (9.5%) patients had clue cells. 25 (12.5%) females had >30 pus cells.

Table 23: MICROSCOPY

MICROSCOPY	NO OF PATIENTS	PERCENTAGE (%)
NORMAL	142	71
PSEUDOHYPHAE AND SPORES	14	7
CLUE CELLS	19	9.5
>30 PUS CELLS	25	12.5
TOTAL	**200**	**100**

Figure 26: MICROSCOPY



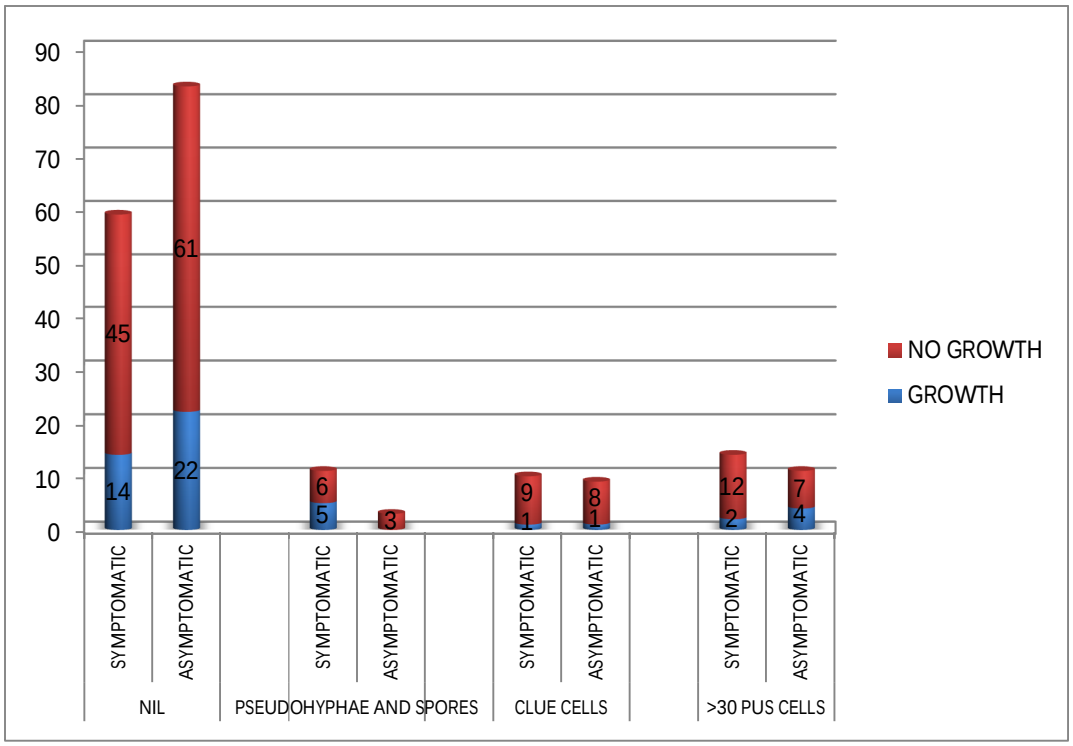
CULTURE AND MICROSCOPY

35.71% (5 out of 14) patients with pseudohyphae and spores on microscopy had growth in culture

Table 24: CULTURE AND MICROSCOPY

MICROSCOPY	NO OF PATIENTS	CULTURE GROWTH	PERCENTAGE (%)
NORMAL	142	36	25.35
PSEUDOHYPHAE AND SPORES	14	5	35.71
CLUE CELLS	19	2	10.53
>30 PUS CELLS	25	6	24

Figure 27: DISTRIBUTION OF MICROSCOPIC FINDINGS AND CULTURE GROWTH AMONG SYMPTOMATICS AND ASYMPTOMATICS



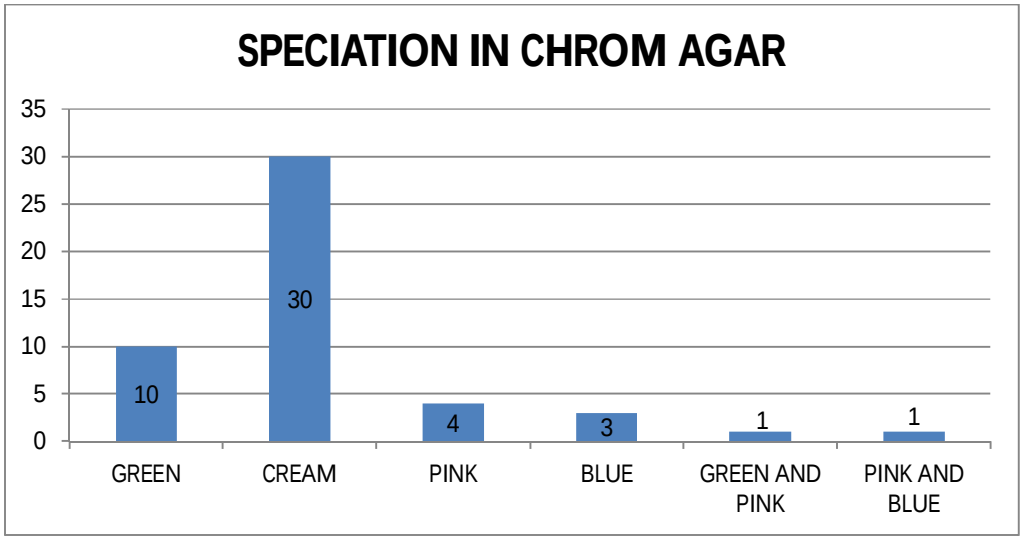
SPECIATION IN CHROM AGAR

C.glabrata was seen in 30 (61.22%) patients. 20.4% culture growth was due to C.albicans and 8.16% was due to C.krusei. C.tropicalis was present in 6.12% of culture growth. 4.08% had mixed infection with either C. albicans and C.krusei or C.tropicalis and C.krusei**.**

Table 25: SPECIATION IN CHROM AGAR

SPECIES	COLOUR	N = 49	PERCENTAGE (%)
C.glabrata	Cream	30	61.22
C.albicans	Green	10	20.4
C.krusei	Pink	4	8.16
C.tropicalis	Blue	3	6.12
C.albicans and C.krusei	Green and Pink	1	2.04
C.tropicalis and C.krusei	Blue and Pink	1	2.04

Figure 28: SPECIATION IN CHROM AGAR



GERM TUBE FORMATION

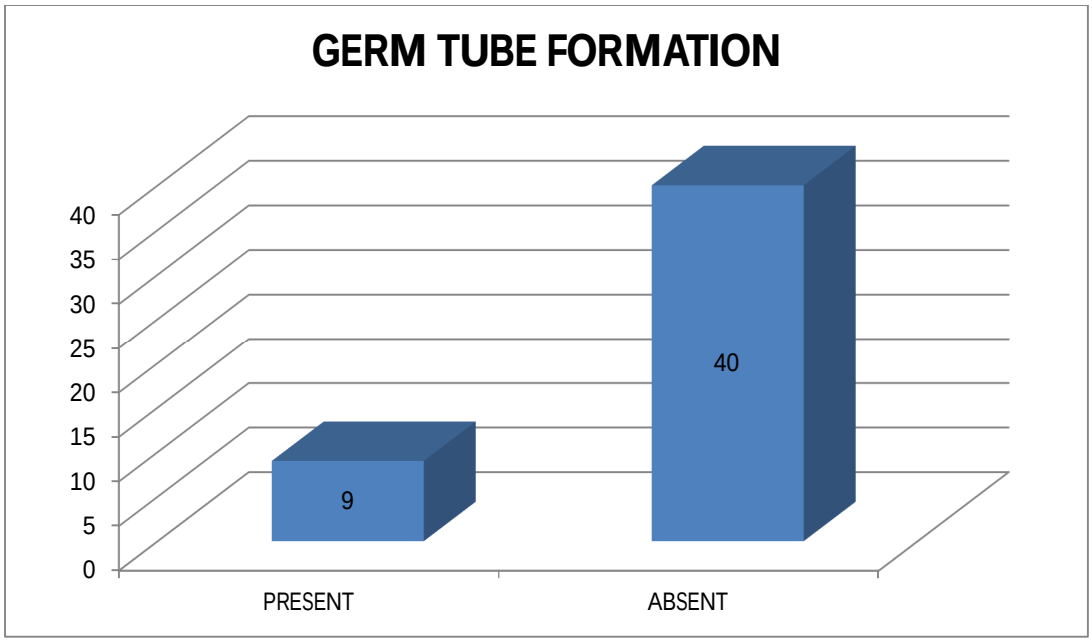
Germ tube formation with incubation in serum was positive in 18.37% (9) isolates.

Germ tube is present in 9 out of 10 patients (90%) who had green colour growth in CHROM Agar.

Table 26: GERM TUBE FORMATION

GERM TUBE	NO OF ISOLATES	PERCENTAGE (%)
PRESENT	9	18.37
ABSENT	40	81.63
TOTAL	49	100

Figure 28: GERM TUBE FORMATION



ANTIFUNGAL SUSCEPTIBILITY PATTERN

Table 27: ANTIFUNGAL SUSCEPTIBILITY PATTERN

ANTIFUNGAL SUSCEPTIBILITY	NO OF STRAINS SUSCEPTIBLE	PERCENTAGE (%)
NYSTATIN	10	20.4
FLUCONAZOLE	2	4.08
MICONAZOLE	3	6.12
NS, FLU	3	6.12
NS, MIC	12	24.48
NS,FLU, MIC	9	18.37
NS, FLU, MIC, CC	3	6.12
NS, MIC, CC	3	6.12
FLU, MIC, CC	2	4.08
MIC, CC	2	4.08
TOTAL	**49**	**100**

EFFECTIVENESS OF INDIVIDUAL ANTIFUNGALS

Nystatin was effective against 40 (81.63%) strains of the 49 isolated. Miconazole has activity against 34 (69.38%), Fluconazole against 19 (38.77%) and Clotrimazole against 10 (20.41%) Candida strains isolated.

Figure 30: EFFECTIVENESS OF INDIVIDUAL ANTIFUNGALS

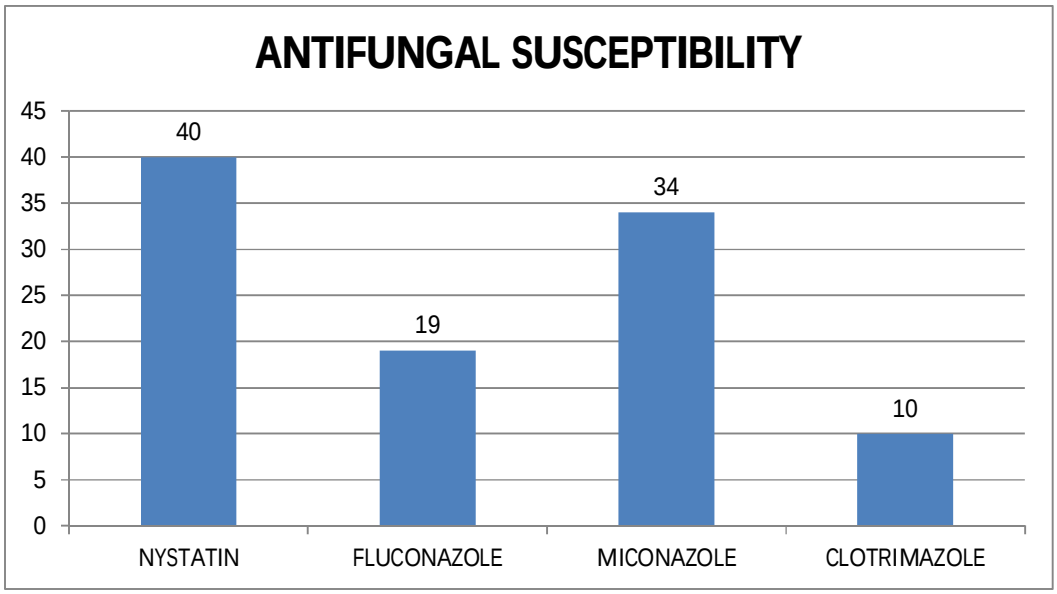


Figure 31: **PERCENTAGE DISTRIBUTION OF ANTIFUNGAL SUSCEPTIBILITY**

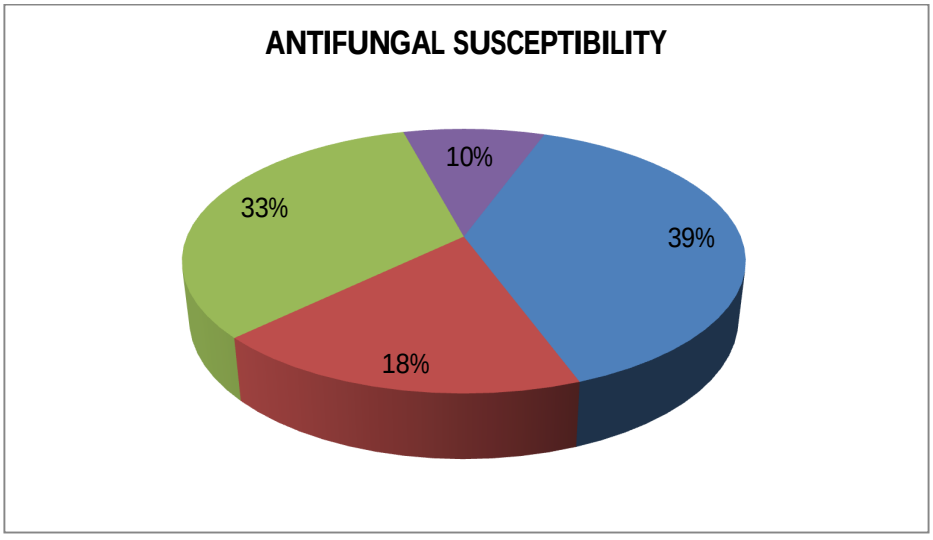


Image 1: Vulva with erythema, white discharge and soddening

Image 2: Speculum examination showing thick curdy white adherent discharge in vagina

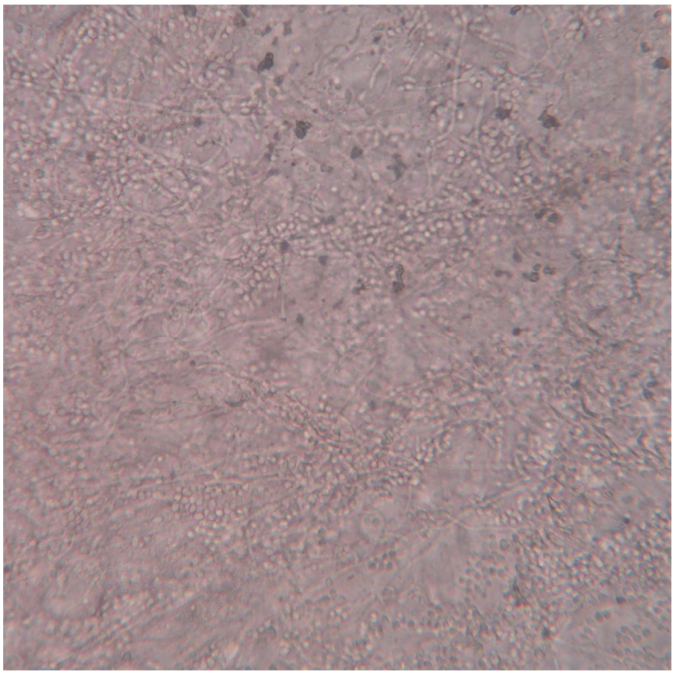


Image 3: KOH mount with pseudohyphae and spores

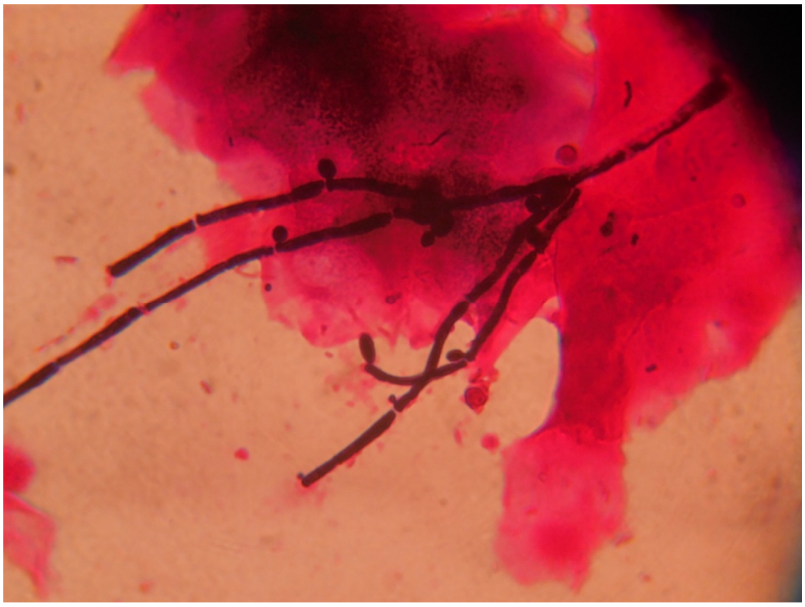


Image 4: Gram's stain showing gram positive pseudohyphae with spores

Image 5: SDA medium with creamy yeasty colonies

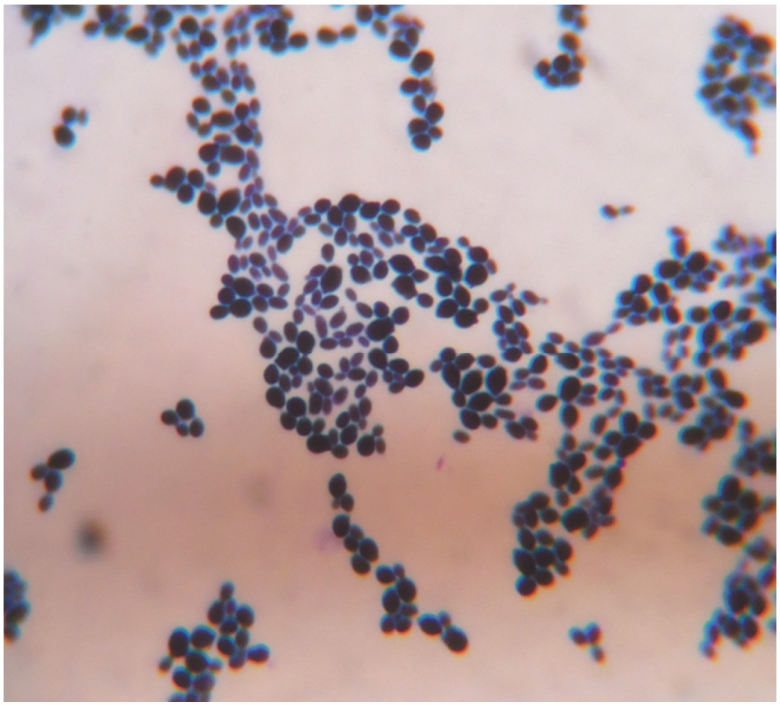


Image 6: Lactophenol cotton blue mount showing positive germ tube production

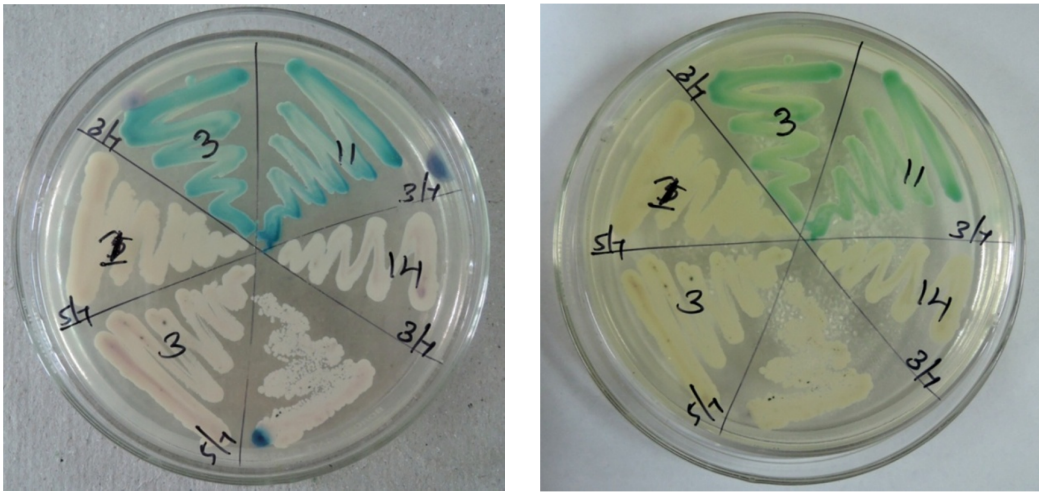


Image 7: Growth on CHROM agar medium with pink, blue, green and cream colour colonies

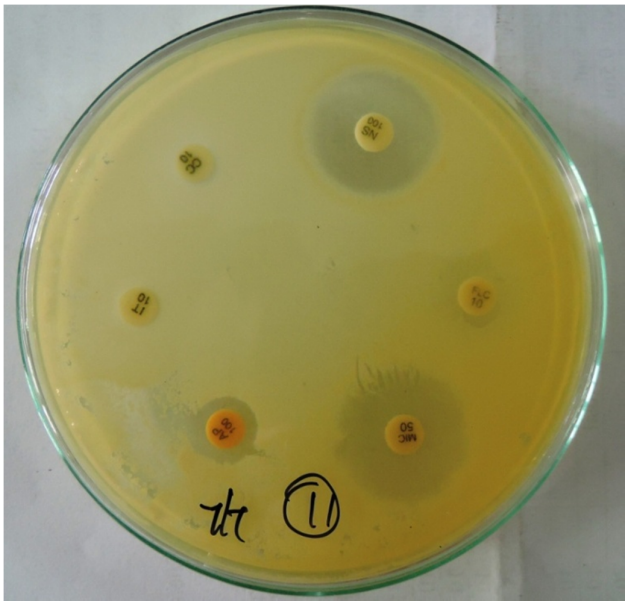


Image 8: Disk diffusion method for antifungal susceptibility testing showing inhibition of growth around miconazole and nystatin discs.

Discussion

DISCUSSION

In this study we included a total of 200 female patients attending STD outpatient department. Of these 106 (53%) patients were asymptomatic females referred from other departments to rule out STDs and 94 (47%) were symptomatic female patients.

58 (29%) patients had Vulvovaginal Candidiasis proven by either culture or microscopy. Of the symptomatic patients, 28 (29.79%) patients had VVC. Of the asymptomatic patients, 30 patients (28.3%) had VVC.

This is less when compared to the study conducted by Bro.F[3]. in Scandinavia in which 32.36% patients had VVC proven by either culture or microscopy. The prevalence of VVC in symptomatic patients was 39% and prevalence of VVC in asymptomatic patients was 22%.

Culture for Candida in Sabouraud's Dextrose Agar was positive in 49 (24.5%) patients. This is similar to studies conducted by Eckert[33] et al where samples from 774 female patients were cultured with 24% (186) culture positivity and by Hopwood where samples from 277 women were cultured with 24% culture positivity. But in a study conducted by Srujana[37] in Obstetrics and Gynaecology department, All India Institute of Medical Sciences, only 18.7% were culture positive among 601 women.

14 (7%) patients had pseudohyphae and spores on examination in Potassium hydroxide mount and in Gram's stain.

This was much less when compared to the studies conducted by Zdolsek[61] et al in Sweden which showed 13.9% smear positivity and Engbert[25] et al which showed 30.41% positive microscopic identification in KOH mount.

44 patients (22%) were only culture positive. 9 patients (4.5%) were only smear positive. 5 patients (2.5%) were both culture and smear positive. A study by Kaplan[10] et al showed that there was no instance in which smear was positive and culture negative which is in contrast to our study. But a study by Sonnex[24] et al showed that 12.36% were smear positive and culture negative.

Of the total symptomatic females, 22.91% were culture positive and of the asymptomatic females, 25.96% were culture positive. This is in contrast to a study by Iara Linhares[41] where 72.7 % of symptomatic females were shown to be culture positive. In a study by J.D.Oriel[2] et al 20% of the asymptomatic females were culture positive comparable to our study.

Thus our study shows similar or greater culture positivity than most comparable studies but less positivity in microscopic examination. The lower culture positivity in symptomatic group in our study could be due to other causes of symptomatic genital infections like Bacterial vaginosis, Trichomoniasis and Cervicitis being more common. About one quarter of the asymptomatic females had positive culture growth showing that Candidal

infection can remain symptom free and culture is one of the sensitive methods in detecting asymptomatic Candidiasis of genital tract.

DEMOGRAPHIC CHARACTERS

AGE

Of our study population of 200, 114 (57%) patients were in the age group from 26 – 40. Maximum number of VVC cases was in the 36- 40 age group (7.5%). In a study by Paulitsch[26] et al, females in the age group of 21 to 40 years were shown to be more prone for Vulvovaginal Candidiasis.

LITERACY

43% of the females in our study population had completed high school, 7% had completed primary school, 9% had higher secondary education and 5% had a college degree. 36% (73 patients) were illiterate. The distribution based on education was almost similar in both symptomatic and asymptomatic group. Prevalence of Vulvovaginal Candidiasis was highest in patients who had completed high school (13.5%).

MARITAL STATUS

Of the 200 participants in our study population 192 (96%) were married and 8 (4%) were unmarried. Vulvovaginal Candidiasis was seen in 27.08% married females and 75% unmarried females.

CONTRACEPTION

150 of the 192 married females (78%) had undergone puerperal sterilisation. 41 females (21%) followed no contraceptive method. They were either planning for first or second pregnancy or were elderly menopausal women not in need of contraception. Barrier method was followed by one female whose husband was HIV positive. None of the females in our study population followed Oral Contraceptive pills or intrauterine contraceptive device. Hence our study group was not comparable to other groups where OCPs and IUCDs were the major predisposing factors studied for risk of Vulvovaginal Candidiasis[7].

RISK FACTORS

The risk factors found in our study were controlled and uncontrolled diabetes, steroid intake, HIV and multiple sexual contacts. 173 females had no known risk factors.

DIABETES

12 (6%) patients were diabetics. 8 of them had diabetes controlled with oral hypoglycemics and blood sugar in the normoglycemic range. 4 were uncontrolled diabetes on insulin therapy with blood sugar levels above 200 mg /dl. 16.66% of the diabetics had Vulvovaginal candidiasis compared to 29.79% in non diabetics. This was in contrast to a study by Sobel[16] et al who found no statistically significant correlation between Candida colonisation and diabetes.

A study by Deepti[14] et al showed that isolation of Candida by culture is similar in both diabetics and non diabetics. However diabetics (67.5%) showed a slightly higher incidence than non diabetics (54.7%)

MULTIPLE SEXUAL CONTACTS

6 (3%) females had multiple sexual contacts with history of frequent unprotected sexual intercourse with unknown persons. 16.66% of sexually promiscuous women had VVC when compared to 29.38% of women with single partners. A study by Geigel showed that frequent vaginal intercourse had no association with increased risk of VVC. In contrast a study by Spinillo[7] et al showed that increased frequency of sexual activity significantly correlated with recurrent infection.

HIV

8 patients (4%) were HIV positive in our study. 4 were on anti retroviral therapy. 4 were recently diagnosed with their immunological and virological status yet to be evaluated. 12.5% of HIV patients had VVC when compared to 29.69% of HIV negatives.

This is in contrast to the study by Spinillo[20] where 61.9% of HIV positive and 32.3% of the HIV negative had culture proven VVC showing increased prevalence of VVC among HIV positive patients. A study by Duerr[19] et al conducted in American college of Obstetrics and Gynaecology showed no increased risk of VVC or rates of colonisation based on HIV serostatus.

STEROID INTAKE

3 patients (1.5%) were on steroids and one of them had vulvovaginal Candidiasis.

SEROLOGICAL STATUS

HIV STATUS

192 patients tested negative and 8 patients were positive. 6 of the HIV positive patients were symptomatic and 2 were asymptomatic. 12.5% of HIV patients had Vulvovaginal Candidiasis.

VDRL STATUS

Only 2 patients were VDRL reactive with one having 1:4 dilutions and the other patient having 1:1 dilution. Both were TPHA positive. Both VDRL reactive patients were asymptomatic females and VVC was present in one of them.

CLINICAL FEATURES

SYMPTOMS

36.17% had vaginal discharge as their sole complaint. 11.7% complained of itching and 35.11% complained of both discharge and itching. 17.02% had other complaints.

12 females (35.29%) who complained of vaginal discharge had Vulvovaginal Candidiasis. 3 females (27.27%) who complained of itching

alone and 8 females (24.24%) who complained of both discharge and itching had VVC. 5 females (31.25%) who had other complaints had VVC.

This is in contrast to the study by Ryan[45] et al which strongly associated vulval pruritis with VVC and not with abnormal vaginal discharge. In studies conducted by Anderson[47] et al and Mycoschaaf[56] et al they showed that symptoms and signs cannot differentiate between causes of vaginitis and are limited in their diagnostic power. But in both studies itching was more commonly associated with VVC than other symptoms.

SIGNS

On examination of the patients, 147 (73.5%) had mild mucoid discharge, 32 (16%) had moderate white discharge, 9 (4.5%) had erythema and maceration of vulva, 8 (4%) had curdy white discharge and 4 (2%) patients had other findings like wart, erosion and vesicles.

27.21% of patients with mild mucoid discharge, 21.88% of moderate white discharge, 44.44% patients with erythema and maceration and 25% patients with other findings had VVC. 75% of patients with curdy white discharge had VVC. Thus curdy white discharge was the most common sign associated with VVC.

This is in contrast with study by Anis Ahmed[39] et al in which most common sign associated with culture positive VVC was vaginal erythema. Another study by Iara[41] et al showed the same results.

Mild mucoid discharge is normally present in most females without producing any symptoms and is not commonly associated with any genital tract infection. But in our study many VVC patients had only mild mucoid discharge on examination without any of the classical findings associated with VVC. And most were asymptomatic. Thus apparently normal females without any signs and symptoms of Vulvovaginal Candidiasis can still harbour the pathogenic fungal organism.

MICROSCOPY

Microscopic examination of vaginal discharge was normal in 142 patients.

A total of 14 (7%) females of the study group had pseudohyphae and spores on microscopic examination. 35.71% (5 out of 14) patients with pseudohyphae and spores on microscopy had growth in culture. Of the 49 culture proven VVC, in our study 5 (10.20%) had pseudohyphae and spores in microscopy.

In a study by Anis Ahmad[39] in Aligarh University, 15.90% of the study population of 1050 women had pseudohyphae and spores on microscopic examination. And of the 215 culture proven VVC, 167 (77.67%) had pseudohyphae and spores in microscopy. A study by Iara[41] et al showed that 87.1% of the culture proven VVC and 5.1% of the culture negative patients had pseudohyphae in microscopy.

Our study has significantly lower microscopic identification rates when compared to the above studies probably because of inadequate material or observer variation.

In our study pseudohyphae and spores were present in both Gram's stain and KOH in all patients. A study by Jean Abbott[40] showed that Gram stain with pseudohyphae and spores was the most accurate laboratory finding in Vulvovaginal Candidiasis and saline and KOH mount were moderately accurate.

19 (9.5%) patients had clue cells out of whom 2 (10.52%) had culture growth. Thus 4.08% of the patients with culture positive VVC and 11.25% culture negative patients had clue cells. This is slightly lower when compared to the study by Iara[41] in which 9.1% of the culture positive and 16.8% of the culture negative females had clue cells.

25 (12.5%) females had >30 pus cells on microscopy out of whom 6 (24%) had culture growth.

CORRELATION BETWEEN CULTURE AND MICROSCOPY IN PATIENTS WITH SYMPTOMS AND SIGNS

Among 28 symptomatic VVC patients, 78.57% were culture positive and 39.29% were smear positive. Among 30 asymptomatic VVC patients, 90% were culture positive and only 10% were smear positive.

In symptomatic patients, 83.33% of VVC patients with discharge had culture growth whereas only 25% were detected on microscopy. Similarly 75% of those with itching, discharge and itching and other complaints had culture growth whereas microscopic identification of Candida was possible in only 50% of those with itching, 62.5% of those with discharge and itching and 25% of those with other complaints.

100% of patients with curdy white discharge were culture positive and 75% were smear positive. 87.5% of those with mild mucoid discharge were culture positive but only 12.5% were smear positive. 85.71% of those with moderate white discharge were culture positive but only 14.29% were smear positive. 25% of with erythema and maceration were culture positive but 100% were smear positive.

Thus patients with the classical curdy white discharge associated with VVC were easily detected by both culture and microscopy. This is similar to the study by Zdolsek[61] et al which showed that microscopy and culture was equally sensitive in patients with classical symptoms and signs.

In patients with mild mucoid discharge and moderate white discharge most of the smears were negative, but culture proved a valuable tool in identifying Candidiasis detecting more than 85% cases.

These results show that if microscopy, which is the commonly used bedside test to confirm Candidiasis, alone is used for diagnosis, most of the VVC cases would be missed. Culture has significantly increased the detection of VVC cases. Culture and microscopy used in combination would be better than either tests used alone for detection of Vulvovaginal Candidiasis.

A study by Zdolsek[61] had concluded along the same lines saying that microscopy may be the first line diagnostic tool but culture should be done whenever there is clinical suspicion in case of a negative smear or when speciation and susceptibility testing is to be done.

But a study by Evans[17] et al showed that C.albicans may be commensal and demonstration by culture does not necessarily confirm and that results should be correlated with signs and symptoms before taking a decision to treat.

SPECIATION IN CHROM AGAR

C.glabrata seen in 30 (61.22%) patients was the most common species isolated. 20.4% culture growth was due to C.albicans, 8.16% was due to C.krusei and 6.12% was due to C.tropicalis. 4.08% had mixed infection with either C. albicans and C.krusei or C.tropicalis and C.krusei. Overall prevalence of non albicans Candida species is 79.59%.

In a study by Srujana[37], yeasts isolated consisted of Candida glabrata (50.4%), C. albicans (35.1%), C. tropicalis (10.8%), C. krusei (2.7%) and C. parapsilosis (0.9%). Overall prevalence of non-albicans candida species was 64.8 per cent which is similar to our study.

In contrast, in a study by Anis ahmad[39] et al Candida albicans accounted for 46.9% of cases, Candida glabrata 36.7%, Candida parapsilosis 10.2%, Candida tropicalis 2.8% and Candida krusei 1.4%.

GERM TUBE FORMATION

Germ tube formation with incubation in serum was positive in 18.37% (9) isolates. 90% of C.albicans showed positive germ tube formation.

ANTIFUNGAL SUSCEPTIBILITY

40 (81.63%) strains were sensitive to Nystatin, 34 (69.38%) strains were sensitive to Miconazole, 19 (38.77%) strains were sensitive to Fluconazole and 10 (20.41%) strains were sensitive to Clotrimazole.

Thus in our study, Nystatin was found to be the most sensitive antifungal followed by miconazole and fluconazole.

30 out of 49 strains (61.22%) were resistant to fluconazole. This is much higher when compared to the study by Richter[38] et al in which only 3.7% of strains were resistant to fluconazole.

79.59% of strains were susceptible to one or more of azoles (fluconazole, miconazole or clotrimazole). This was slightly lower than in Richter's study in which 94.3% of strains were susceptible to one or more azoles.

Summary

SUMMARY

In this study out of 200 female patients,

- 106 (53%) were asymptomatic and 94 (47%) were symptomatic.
- Of the symptomatic patients, 28 (29.79%) patients had VVC.
- Of the symptomatic patients, 30 patients (28.3%) had VVC.
- Vulvovaginal Candidiasis proven by culture was seen in 49 (24.5%) patients.
- Microscopic identification of Candida was seen in 14 (7%) patients.
- VVC proven by either culture or microscopy was seen in 58 (29%) patients.
- 5 patients (2.5%) were both culture and smear positive.

DEMOGRAPHIC CHARACTERS

AGE

The most common age group of the females in our study is 26 – 40 years with 114 patients (57%) in this group. Maximum number of VVC patients was in the 36- 40 age group (7.5%)

EDUCATION

Majority (43%) of the females in our study population had completed high school. Prevalence of Vulvovaginal Candidiasis was highest in patients who had completed high school (13.5%).

MARITAL STATUS

192 (96%) participants in our study were married. Vulvovaginal Candidiasis was seen in 27.08% married females and 75% unmarried females.

CONTRACEPTION

78.13% had undergone puerperal sterilisation. 21.35% followed no contraceptive method. Barrier method was followed by one female. 22.67% of those who had undergone puerperal sterilisation and 19.51% of those who followed no contraception had VVC.

RISK FACTORS

171 (85.5%) females had no known risk factors.

DIABETES

The total number of diabetics in our study was 12 (6%). 16.66% of the diabetics had Vulvovaginal candidiasis compared to 25% in non diabetics.

MULTIPLE SEXUAL EXPOSURES

The number of females with multiple sexual contacts and frequent unprotected sexual intercourse was 6 (3%). 16.66% of sexually promiscuous women had VVC.

HIV

The prevalence of HIV patients in our study was 8(4%). 12.5% of HIV patients had VVC.

STEROID INTAKE

The prevalence of patients taking steroids in our study was 3(1.5%). 33.33% of patients on steroids had VVC.

SEROLOGICAL STATUS

HIV STATUS

192 patients tested negative and 8 (4%) patients were positive. 12.5% of HIV patients had Vulvovaginal Candidiasis.

VDRL STATUS

2 (1%) patients were VDRL reactive. 50% of VDRL reactive patients had Vulvovaginal Candidiasis.

CLINICAL FEATURES

SYMPTOMS

Vaginal discharge – 36.17% had vaginal discharge as their sole complaint. 35.29% (12) patients who complained of vaginal discharge had VVC.

Itching – 11.7% complained of itching. 27.27 % (3) patients who complained of itching had VVC.

Vaginal discharge and itching – 35.11% complained of both discharge and itching. 24.24% (8) of these patients had VVC.

Other complaints – 17.02% had other complaints like dysuria, dyspareunia, soreness or ulcer. 31.25% (5) who had other complaints had VVC.

SIGNS

Mild mucoid discharge – 147 (73.5%) patients had mild mucoid discharge. 27.21% of patients with mild mucoid discharge had VVC.

Moderate white discharge – 32 (16%) patients had moderate amount of white discharge. 21.88% of those with moderate white discharge had VVC.

Curdy white discharge – 8 (4%) patients had curdy white discharge. 75% of patients with curdy white discharge had VVC.

Erythema and maceration – 9 (4.5%) patients had erythema and maceration of vulva. 44.44% of these patients had VVC.

Other findings – 4 (2%) patients had other findings like wart, erosion and vesicles. 25% patients with other findings had VVC.

MICROSCOPY

Microscopic examination of vaginal discharge was normal in 142 patients.

Pseudohyphae and spores - 14 (7%) females of the study group had pseudohyphae and spores. 5 (35.71%) patients had culture positivity.

Clue cells - 19 (9.5%) patients had clue cells out of whom 2 (10.53%) had culture growth.

>30 pus cells - 25 (12.5%) females had >30 pus cells on microscopy out of whom 6 (24%) had culture growth.

SPECIATION IN CHROM AGAR

C.glabrata present in 61.22% of the culture growth was the most common species isolated.

C.albicans	-	20.4%
C.krusei	-	8.16%
C.tropicalis	-	.12%
Mixed infection	-	4.08%

GERM TUBE FORMATION

Germ tube was positive in 18.37% (9 out of 49) isolates.

90% of C.albicans showed positive germ tube formation.

ANTIFUNGAL SUSCEPTIBILITY

Nystatin which was sensitive in 40 (81.63%) strains was the most effective antifungal in our study.

Miconazole	-	34 (69.38%)
Fluconazole	-	19 (38.77%)
Clotrimazole	-	10 (20.41%).

Conclusion

CONCLUSION

- In our study of the total 200 patients, 94 (47%) were symptomatic and 106 (53%) were asymptomatic.
- The prevalence of Vulvovaginal Candidiasis proven by either culture or microscopy was 29% (58 patients).

- Of the symptomatic patients,
    - The prevalence of VulvovaginalCandidiasis based on culture was 23.40% (22 patients)
    - The prevalence of VulvovaginalCandidiasis based on microscopy was 11.7% (11 patients)
    - The prevalence of VulvovaginalCandidiasis by culture and microscopy was 5.32% (5 patients)

- Of the asymptomatic patients,
    - The prevalence of Vulvovaginal Candidiasis based on culture was 25.47% (27 patients)
    - The prevalence of Vulvovaginal Candidiasis based on microscopy was 2.83% (3 patients)
    - The prevalence of Vulvovaginal Candidiasis by culture and microscopy was nil

- Highest prevalence of VVC patients was seen in 36 - 40 age group.
- 96% of our study population were married women.
- The participants had undergone either puerperal sterilisation or followed no contraception. This had no implication on the risk of VVC. Known risk factors like Oral contraceptive pills and Intrauterine Contraceptive Device were practiced by none.
- Risk factors like HIV, diabetes and steroid intake did not have any effect on the prevalence of VVC.
- 8 patients were HIV positive and 2 were VDRL reactive.
- The most common symptom was vaginal discharge. Prevalence of VVC was highest in those who complained of vaginal discharge (35.29%). Patients with vaginal discharge showed maximum culture positivity (83.33%). Those with discharge and itching showed maximum smear positivity (62.5%).
- The most common clinical finding on examination was mild mucoid discharge. Maximum culture positivity was seen in patients with curdy white discharge (100%). Maximum smear positivity was seen in patients with erythema and maceration (100%).
- 71% had normal microscopy. Most common microscopic finding was >30 pus cells (12.5%).

- C.glabrata seen in 30 patients (61.22%) was the most common species isolated followed by C.albicans (20.4%).
- Germ tube was positive in 18.37% isolates.
- Nystatin was effective against 40 strains (81.63%) and was the antifungal to which Candida had maximum susceptibility followed by Miconazole and Fluconazole.

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Annexures

ABBREVIATIONS USED

STD	–	Sexually Transmitted Disease
VVC	–	Vulvo Vaginal Candidiasis
HIV	–	Human Immunodeficiency Virus
VDRL	–	Venereal Disease Research Laboratory
TPHA	–	Treponema pallidum Hemagglutination Assay
CHROM	–	Chromogenic
FLU	–	Fluconazole
NS	–	Nystatin
MIC	–	Miconazole
CC	–	Clotrimazole
SDA	–	Sabouraud's dextrose agar
CMA	–	Corn Meal Agar
KOH	–	Pottasium hydroxide
OCP	–	Oral Contraceptive Pill
IUCD	–	IntraUterine Contraceptive Device
SAP	–	Secreted Aspartyl Proteinases
CMI	–	Cell-mediated immunity
IL	–	Interleukin
BV	–	Bacterial vaginosis
RVVC	–	Recurrent Vulvo Vaginal Candidiasis
SNP	–	Single Nucleotide Polymorphisms
PCR	–	Polymerase Chain Reaction

DNA	–	Deoxy Ribonucleic acid
EIA	–	Enzyme Immuno Assay
CLSI	–	Clinical and laboratory standard institute
MIC	–	Minimum Inhibitory Concentration
S-DDa	–	Susceptible dose dependent

MASTER CHART

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
1	29	M	dip	nil	itching	nil	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
2	38	M	8th	PS	discharge	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
3	40	M	ill	PS	discharge,itching,dysuria	nil	mild mucoid	PC	G	A	G P	NS,CC,Mic	N	NR
4	40	M	ill	PS	discharge,itching,dysuria	nil	mild mucoid	PC	NG	NA	NA	NA	N	NR
5	20	S	10th	NA	discharge and itching	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
6	30	M	8th	PS	discharge and itching	nil	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
7	18	S	12th	NA	discharge	STE	erythema, soddening,intertrigo	PHNS	NG	NA	NA	NA	N	NR
8	30	M	10th	Barrier	discharge and itching	HIV on ART	moderate white discharge	>30 PC	NG	NA	NA	NA	P	NR
9	32	M	8th	nil	discharge and itching	nil	soddening, maceration	PHNS	NG	NA	NA	NA	P	NR
10	50	M	ill	PS	discharge	nil	mild mucoid	PC	NG	NA	NA	NA	N	NR
11	24	M	10th	nil	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	P	NR
12	40	M	ill	PS	discharge	nil	curdy white discharge	PHNS	G	P	G	NS	N	NR
13	21	M	dip	nil	itching, dysuria, soreness	nil	soddening, maceration	nil	NG	NA	NA	NA	N	NR
14	32	M	8th	nil	discharge and itching	nil	soddening, maceration	nil	NG	NA	NA	NA	N	NR
15	30	M	8th	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
16	30	M	ill	nil	discharge and itching	DM U	mild mucoid	nil	NG	NA	NA	NA	N	NR
17	33	M	6th	PS	discharge,itching,dysuria	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
18	27	M	10th	nil	discharge and itching	EMC	mild mucoid	PC	NG	NA	NA	NA	N	NR
19	43	M	7th	PS	itching	EMC	mild mucoid	nil	NG	NA	NA	NA	N	NR
20	25	M	10th	nil	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
21	35	M	ill	PS	discharge	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
22	27	M	ill	PS	discharge and dysuria	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
23	28	M	6th	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
24	50	M	ill	PS	itching	DM	mild mucoid	nil	NG	NA	NA	NA	N	NR
25	43	M	5th	PS	discharge and itching	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
26	39	M	ill	PS	discharge and soreness	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
27	31	M	12th	PS	itching	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
28	50	M	4th	PS	itching	DM	mild mucoid	nil	G	A	P	NS,mic	N	NR
29	21	M	ill	PS	discharge	nil	mild mucoid	nil	G	P	G	NS,mic,flu	N	NR
30	50	M	ill	PS	discharge	nil	moderate white discharge	nil	G	A	B	mic	N	NR
31	37	M	ill	PS	discharge,dyspareunia	nil	mild mucoid	nil	G	A	C	NS,mic	N	NR
32	44	M	12th	PS	discharge	nil	mild mucoid	nil	G	A	C	NS	N	NR
33	26	M	12th	nil	discharge and itching	nil	mild mucoid	nil	G	A	C	flu,mic,NS,CC	N	NR
34	33	M	ill	PS	itching	nil	curdy white discharge	nil	G	A	C	NS,CC,Mic	N	NR
35	40	M	ill	PS	discharge and itching	nil	curdy white discharge	PHNS	G	A	G	NS	N	NR
36	52	M	ill	PS	discharge and dysuria	nil	mild mucoid	nil	G	A	C	NS	N	NR
37	39	M	5th	PS	discharge	nil	mild mucoid	PC	G	A	C	flu,mic,NS	N	NR
38	35	M	10th	PS	discharge	nil	moderate white discharge	CC	G	P	G	NS,mic	N	NR
39	38	M	8th	PS	itching and soreness	nil	erythema,maceration	PHNS	G	A	C	flu,NS	N	NR
40	40	M	ill	nil	discharge	nil	mild mucoid	nil	G	A	C	NS	N	NR
41	37	M	12th	PS	discharge	nil	mild mucoid	PC	G	A	C	flu	N	NR
42	33	M	6th	PS	discharge,dysuria,ulcer	nil	curdy white discharge,erosion	nil	G	A	C	NS,flu	N	NR
43	51	M	ill	PS	dysuria	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
44	50	M	ill	PS	itching	DM	mild mucoid	nil	NG	NA	NA	NA	N	NR
45	29	M	ill	nil	discharge and itching	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
46	39	M	10th	PS	itching	nil	mild mucoid, intertrigo	nil	NG	NA	NA	NA	N	NR
47	38	M	8th	PS	discharge	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
48	23	M	12th	nil	discharge and itching	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
49	49	M	ill	nil	discharge	EMC, HIV	mild mucoid	nil	NG	NA	NA	NA	P	NR
50	39	M	ill	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
51	29	M	8th	PS	discharge and itching	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
52	35	M	ill	PS	discharge	HIV on ART	mild mucoid, wart	nil	NG	NA	NA	NA	P	NR
53	45	M	ill	PS	itching	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
54	32	M	10th	PS	discharge,itching,dysuria	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
55	38	M	5th	PS	discharge and soreness	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
56	27	M	9th	PS	discharge,itching,dysuria	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
57	30	M	10th	PS	discharge	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
58	47	M	ill	nil	discharge	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
59	24	M	ill	nil	itching,raised genital lesion	EMC	mild mucoid, wart	PC	NG	NA	NA	NA	N	NR
60	40	M	10th	PS	discharge and itching	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
61	33	M	10th	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
62	40	M	ill	PS	discharge and itching	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
63	36	M	ill	nil	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
64	40	M	ill	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
65	41	M	6th	PS	discharge	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
66	28	M	ill	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
67	40	M	8th	PS	discharge and itching	nil	curdy white discharge	nil	NG	NA	NA	NA	N	NR
68	39	M	8th	PS	discharge and itching	nil	curdy white discharge	nil	NG	NA	NA	NA	N	NR
69	30	M	6th	nil	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
70	44	M	ill	PS	discharge and itching	EMC	moderate white discharge	CC	NG	NA	NA	NA	N	NR
71	52	M	ill	PS	itching	nil	soddening, maceration	nil	NG	NA	NA	NA	N	NR
72	38	M	ill	PS	discharge and itching	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
73	33	M	8th	nil	dysuria	nil	mild mucoid	PHNS	NG	NA	NA	NA	N	NR
74	51	M	ill	PS	discharge and itching	nil	mild mucoid	nil	G	A	P	NS, mic	N	NR
75	23	S	dip	NA	discharge and itching	nil	curdy white discharge	PHNS	G	A	C	flu, NS	N	NR
76	25	M	8th	PS	discharge	nil	moderate white discharge	>30 PC	G	A	B	Flu,mic, CC	N	NR
77	35	M	5th	PS	discharge	nil	moderate white discharge	>30 PC	G	A	C	NS,CC,Mic	N	NR
78	20	S	deg	NA	discharge and itching	nil	curdy white discharge	PHNS	G	A	C	flu	N	NR
79	28	M	10th	nil	discharge and itching	nil	mild mucoid	PC	NG	NA	NA	NA	N	NR
80	35	M	9th	PS	discharge and dysuria	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
81	20	M	12th	nil	discharge	nil	moderate white discharge	nil	NG	NA	NA	NA	N	NR
82	50	M	10th	PS	discharge	nil	moderate white discharge	PHNS, CC	NG	NA	NA	NA	N	NR
83	39	M	ill	nil	discharge and itching	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
84	35	M	ill	PS	discharge and dysuria	nil	moderate white discharge	PC	NG	NA	NA	NA	N	NR
85	27	M	10th	PS	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
86	53	M	8th	PS	dysuria	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
87	33	M	10th	PS	discharge and itching	nil	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
88	24	M	3rd	PS	discharge and itching	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
89	50	M	ill	PS	itching	nil	mild mucoid	PHNS	NG	NA	NA	NA	N	NR
90	26	S	11th	nil	discharge and itching	nil	soddening, maceration	PHNS	NG	NA	NA	NA	N	NR
91	56	M	7th	PS	discharge and itching	nil	soddening, maceration	nil	NG	NA	NA	NA	N	NR
92	46	M	6th	nil	dysuria	nil	mild mucoid	CC	NG	NA	NA	NA	N	NR
93	30	S	deg	nil	discharge	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
94	48	M	ill	PS	vesicles	HIV on ART	multiple erosions, vesicles	nil	NG	NA	NA	NA	P	NR
95	37	M	8th	nil	nil	nil	mild mucoid	PHNS	NG	NA	NA	NA	N	NR
96	32	M	10th	PS	nil	nil	mild mucoid	nil	G	P	G	NS,mic	N	NR
97	43	M	6th	PS	nil	nil	mild mucoid	nil	G	P	G	flu,mic,NS,CC	N	NR
98	35	M	8th	PS	nil	DM U	mild mucoid	nil	G	A	C	NS,mic	N	NR
99	32	M	ill	PS	nil	nil	mild mucoid	nil	G	A	C	NS,mic	N	NR
100	47	M	ill	PS	nil	nil	mild mucoid	>30 PC	G	A	C	NS, flu, mic	N	NR
101	26	M	4th	PS	nil	nil	mild mucoid	>30 PC	G	P	G	flu,NS,CC,Mic	N	NR
102	41	M	8th	nil	nil	nil	mild mucoid, wart	nil	G	P	G	NS,Mic	N	NR
103	25	M	9th	nil	nil	nil	mild mucoid	>30 PC	G	P	G	NS,Mic	N	NR
104	27	M	5th	PS	nil	nil	mild mucoid	nil	G	A	C	mic	N	NR
105	42	M	ill	PS	nil	nil	mild mucoid	nil	G	A	P	mic,CC	N	NR
106	52	M	ill	PS	nil	nil	mild mucoid	nil	G	A	P B	mic,CC	N	NR
107	44	M	8th	PS	nil	nil	mild mucoid	nil	G	A	C	NS, mic	N	NR
108	28	M	10th	PS	nil	nil	mild mucoid	nil	G	A	C	NS	N	NR
109	37	M	8th	PS	nil	nil	mild mucoid	PC	G	A	C	NS	N	NR
110	38	M	8th	PS	nil	nil	mild mucoid	PC	G	A	C	NS	N	NR
111	37	M	10th	PS	nil	nil	mild mucoid	PC	G	A	C	NS, flu, mic	N	NR
112	60	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
113	60	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
114	30	M	ill	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	R 1:4

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
115	35	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
116	41	M	ill	PS	nil	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
117	38	M	2nd	PS	nil	DM	mild mucoid	nil	NG	NA	NA	NA	N	NR
118	29	M	12th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
119	34	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
120	50	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
121	57	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
122	45	M	ill	PS	nil	nil	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
123	27	M	deg	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
124	40	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
125	35	M	10th	nil	nil	nil	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
126	32	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
127	29	M	dip	PS	nil	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
128	32	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
129	43	M	ill	PS	nil	EMC	mild mucoid	nil	NG	NA	NA	NA	N	NR
130	42	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
131	39	M	12th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
132	60	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
133	22	M	ill	PS	nil	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
134	47	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
135	53	M	ill	PS	nil	DM U	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
136	47	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
137	22	M	6th	nil	nil	nil	moderate white discharge	>30 PC	NG	NA	NA	NA	N	NR
138	50	M	8th	nil	nil	DM	soddening, maceration	nil	NG	NA	NA	NA	N	NR
139	55	M	ill	PS	nil	DM	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
140	24	M	12th	nil	nil	nil	mild mucoid	CC	NG	NA	NA	NA	N	NR
141	27	M	10th	PS	nil	nil	mild mucoid	CC	NG	NA	NA	NA	N	NR
142	55	M	ill	PS	nil	DM	mild mucoid	nil	NG	NA	NA	NA	N	NR
143	35	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
144	33	M	ill	nil	nil	STE	mild mucoid	nil	NG	NA	NA	NA	N	NR
145	30	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
146	32	M	10th	nil	nil	nil	mild mucoid	CC	NG	NA	NA	NA	N	NR
147	51	M	12th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
148	22	M	11th	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
149	60	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
150	29	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
151	28	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
152	40	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
153	28	M	12th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
154	29	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
155	45	M	ill	PS	nil	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
156	36	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
157	37	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
158	23	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
159	43	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
160	47	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
161	56	M	8th	nil	nil	DM	mild mucoid	nil	NG	NA	NA	NA	N	NR
162	32	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
163	55	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
164	60	M	8th	PS	nil	DM U	mild mucoid	>30 PC	NG	NA	NA	NA	N	NR
165	60	M	ill	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
166	31	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
167	49	M	6th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
168	40	M	10th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
169	28	M	12th	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
170	60	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
171	36	M	6th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
172	29	M	10th	PS	nil	nil	mild mucoid	nil	G	A	C	NS, flu, mic	N	NR

SL NO	AGE	MAR	EDU	CON	SYMPTOMS	RF	CF	MICRO	CUL	GT	CHR	AS	HIV	VDRL
173	35	M	ill	PS	nil	nil	mild mucoid	nil	G	A	P	NS, mic	N	NR
174	38	M	10th	PS	nil	nil	mild mucoid	nil	G	A	B	flu, mic, CC	N	NR
175	27	M	10th	nil	nil	nil	moderate white discharge	>30 PC	G	A	C	NS, flu, mic	N	NR
176	32	M	12th	PS	nil	nil	moderate white discharge	>30 PC,CC	GG	A	C	NS, mic	N	R 1:1
177	40	M	7th	PS	nil	nil	mild mucoid	nil	G	A	C	NS	N	NR
178	42	M	8th	PS	nil	nil	mild mucoid	nil	G	A	C	NS	N	NR
179	45	M	10th	PS	nil	EMC	mild mucoid	nil	G	A	C	NS, flu, mic	N	NR
180	19	S	deg	NA	nil	nil	mild mucoid	nil	G	A	C	NS, flu, mic	N	NR
181	33	M	8th	PS	nil	nil	mild mucoid	nil	G	A	C	NS, flu, mic	N	NR
182	56	M	6th	PS	nil	nil	mild mucoid	nil	G	A	C	mic	N	NR
183	33	M	dip	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
184	40	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
185	47	M	ill	PS	nil	nil	moderate white discharge	CC	NG	NA	NA	NA	N	NR
186	36	M	10th	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
187	30	M	8th	PS	nil	nil	mild mucoid	CC	NG	NA	NA	NA	N	NR
188	30	M	8th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
189	47	M	ill	PS	nil	HIV on ART	mild mucoid	nil	NG	NA	NA	NA	P	NR
190	56	M	ill	PS	nil	STE	mild mucoid	nil	NG	NA	NA	NA	N	NR
191	44	M	5th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
192	28	M	7th	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
193	35	M	ill	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
194	27	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
195	50	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
196	53	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
197	60	M	ill	PS	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
198	26	M	10th	nil	nil	nil	mild mucoid	nil	NG	NA	NA	NA	N	NR
199	38	M	10th	PS	nil	HIV	mild mucoid	PHNS	NG	NA	NA	NA	P	NR
200	19	S	12th	NA	nil	nil	mild mucoid	PHNS	NG	NA	NA	NA	N	NR

KEY FOR MASTER CHART

MARITAL STATUS

S - SINGLE

M - MARRIED

EDUCATION

ILL - ILLITERATE

DIP - DIPLOMA

DEG - DEGREE

CONTRACEPTION

PS - PUERPERAL STERILISATION

NIL - NO METHOD FOLLOWED

NA - NOT APPLICABLE

RISK FACTORS

EMC - EXTRA MARITAL CONTACT

HIV - PATIENT WITH HIV INFECTION

HIV ON ART - PATIENT WITH HIV ON ANTIRETROVIRAL TREATMENT

DM - DIABETES MELLITUS

DM U - DIABETES MELLITUS UNCONTROLLED

STE - STEROIDS

NIL - NO RELEVANT RISK FACTORS

MICROSCOPY

PHNS - PSEUDOHYPHAE AND SPORES

CC - CLUE CELLS

PC - PUS CELLS

> 30 PC - >30 PUS CELLS

NIL - NORMAL

CULTURE

G	- GROWTH
NG	- NO GROWTH

GERM TUBE

P	- PRESENT
A	- ABSENT
NA	- NOT APPLICABLE

CHROME AGAR

C	- CREAM
G	- GREEN
B	- BLUE
P	- PINK
NA	- NOT APPLICABLE

ANTIFUNGAL SUSCEPTIBILITY

NS	- NYSTATIN
FLU	- FLUCONAZOLE
MIC	- MICONAZOLE
CC	- CLOTRIMAZOLE
NA	- NOT APLICABLE

HIV STATUS

P	- POSITIVE
N	- NEGATIVE

VDRL REACTIVITY

R	- REACTIVE
NR	- NON REACTIVE

PROFORMA

Name: **Age/ Sex:**

Occupation:

Address: **OP no/ Patient ID no:**

Complaints:

H/o present illness:

H/o vaginal / urethral discharge: type, amount, smell and other characters and relation to menstrual cycle

H/o pruritis :

H/o burning sensation or soreness of vagina:

H/o dysuria :

H/o abdominal pain :

H/o dyspareunia :

H/o fever :

Menstrual history :

Marital History : Single/ married/ divorced/ widow

Living together or alone:

Sexual history :

Last marital contact :

Premarital contact :

Extra marital contact:

Previous sexually transmitted infections / treatments:

Obstetric history :

Past History :

Similar illness in past and treatment:

Tuberculosis :

Diabetes : under control/ not

Hypertension :

Bronchial asthma :

Previous surgeries :

Blood transfusions :

Jaundice :

Antibiotic use : type of antibiotic and duration

OCP or hormonal medication:

IUD insertion :

Family History :

Complaints in partner

Personal History:

Aberrant sexual practices

Tampon use

General examination:

Built :

Pallor :

Jaundice :

Pedal edema :

Generalised lymphadenopathy :

Pulse:

BP:

Systemic examination:

CVS :

RS :

Abdomen :

CNS :

Local examination*:*

Any significant inguinal lymphadenopathy :

Inspection :

Vulva : soddening, maceration, fissures

Vaginal discharge : type, color, amount, smell

Genital ulcers :

Any genital abnormalities:

Per vaginal examination : Position of cervix and uterus

Cervical motion tenderness

Per speculum examination :

Vaginal discharge

Vaginal walls – erythema, erosions or fissures, ulcers

Cervical discharge

Cervical erosion

Skin :

Other Mucosa :

Bones and Joints :

Investigations

Urine routine :

Vaginal / cervical discharge: Grams stain/ wet mount with normal saline and KOH

Ulcers/erosions: Tzanck smear/ Dark field microscopy/ Grams stain

Cervical Culture for Gonococci

VDRL for Syphilis

HIV serology

Swab for Candida culture, CHROM agar culture, antifungal susceptibility testing.

Diagnosis:

Clinical:

Microbiological:

INFORMATION SHEET

TITLE: **"A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT"**

Name of Investigator: Name of Participant:

Purpose of Research: The purpose of the study is to identify the prevalence of candida in symptomatic and asymptomatic female patients, isolation and study the sensitivity of candida to commonly used antifungals.
Study Design: Prospective Observational Study
Study Procedures: Detailed history will be documented. Patient will be subjected to routine clinical examination and vaginal secretion will be collected for microscopy, culture and further tests. Blood will be taken for VDRL and HIV testing. Results will be evaluated and appropriate treatment given.

Possible Risks: No risks to the patient
Possible benefits
To patient: Asymptomatic patients with candida infection are screened and treated. Treatment is given or altered after proven efficacy in case of resistant infections.

To doctor & to other people: Identification of prevalence of non candida species has therapeutic and prognostic significance. Susceptibility testing allows appropriate antifungal therapy. This will help in providing better and complete treatment to other patients in future.

Confidentiality of the information obtained from you: The privacy of the patients in the research will be maintained throughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared

Can you decide to stop participating in the study: Taking part in this study is voluntary. You are free to decide whether to participate in this study or to withdraw at any time

How will your decision to not participate in the study affect you: Your decision will not result in any loss of benefits to which you are otherwise entitled.

Signature of Investigator Signature of Participant
Date :
Place :

PATIENT CONSENT FORM

Title of the study : "**A STUDY ON THE PREVALENCE, ISOLATION AND SENSITIVITY PATTERN OF GENITAL CANDIDA SPECIES IN FEMALE PATIENTS ATTENDING STD OUTPATIENT DEPARTMENT".**

Name of the participant :
Name of the principal investigator : Dr. Shanmuga Priya. K
Name of the Institution : Institute of Venereology, Madras Medical College & Rajiv Gandhi Government General Hospital, Chennai – 3.

Documentation of the informed consent*:*
I _____have read the information in this form (or it has been read to me). I was free to ask any questions and they have been answered. I am over 18 years of age and exercising my free power of choice, hereby consent to be included as a participant in the study.

1. I have read and understood this consent form and the information provided to me
2. I have had the consent document explained to me
3. I have been explained about the nature of the study
4. My rights and responsibilities have been explained to me by the investigator
5. I have been advised about the risks associated with my participation in this study
6. I have been informed the investigator of all the treatments I am taking or have taken in the past _____ months including any native (alternative) treatment
7. I agree to co operate with the investigator and I will inform him/her immediately if I suffer unusual symptoms
8. I have not participated in any research study at any time
9. I am aware of the fact that I can opt out of the study at any time without having to give any reason and this will not affect my future treatment in this hospital
10. I hereby give permission to the investigator to release the information obtained from me as a result of participation in this study to the sponsors, regulatory authorities, Government agencies and institutional ethics committee. I understand that they are publicly presented.
11. I have understand that my identity will be kept confidential if my data are publicly presented
12. I have had my questions answered to my satisfaction.
13. I have decided to be in the research study.

I am aware that if I have any question during the study, I should contact at one of the addresses listed above. By signing this consent form I attest that

the information given in this document has been clearly explained to me and apparently understood by me, I will be given a copy of this consent document.

Participant initials:

For adult participants:

Name and signature/ thumb impression of the participant (or legal representative if participant incompetent)

_____	_____	----------
Name	Signature	Date

Name and signature of impartial witness (required for illiterate patients):

_____	_____	_____
Name	Signature	Date

Address and contact number of the impartial witnesss:

Name and signature of the investigator or his representative obtaining consent:

_____	_____	_____
Name	Signature	Date

ஆராய்ச்சி தகவல் தாள்

தலைப்பு : பெண்கள் பிறப்புறுப்பில் ஏற்படும் கேன்டிடியாஸிஸ் நோயை கண்டறிதல் பற்றிய ஆய்வு.

பங்கேற்பாளரின் பெயர் :

ஆராய்ச்சியாளர் பெயர் :

எனது ஆராய்ச்சி நிறுவன நெறிமுறைகளுக்கு உட்பட்டது என்பதை உறுதி செய்கிறேன்.

கேன்டிடா எனும் பூஞ்சை கிருமியின் தாக்குதலால் பெண்களின் பிறப்புறுப்பில் ஏற்படும் நோயை நுண்ணுயிர் நோக்குதல், நுண்ணுயிர் வளர்ச்சி போன்ற சில பரிசோதனைகள் மூலம் கண்டறிவதே இவ்வாராய்ச்சியின் நோக்கமாகும்.

இதற்கு பெண்களின் பிறப்புறுப்பின் வெளியேற்றம் பரிசோதனை செய்யப்படும். கேன்டிடியாசிஸ் நோயை ஆரம்ப காலகட்டங்களில் கண்டறிவதால் பல பின்விளைவுகளை தவிர்க்கலாம்.

நோயாளிகள் விருப்பத்தின் பேரில் ஆராய்ச்சியில் இணைக்கப்படுவர். விருப்பமில்லை என்றால் எந்நேரமும் விலகிக்கொள்ளலாம்.

ஆராய்ச்சியாளரின் கையொப்பம் பங்கேற்பவரின் கையொப்பம்

தேதி :

ஆராய்ச்சி ஒப்புதல் கடிதம்

தலைப்பு : பெண்கள் பிறப்புறுப்பில் ஏற்படும் கேண்டிடியாஸிஸ் நோயை கண்டறிதல் பற்றிய ஆய்வு.

பெயர் : தேதி :
வயது : நோயாளி எண் :
பால் : ஆராய்ச்சி சேர்க்கை எண் :

நான் ஒப்புதல் படிவத்தை நன்கு படித்து, ஆய்வாளர் ..... உடன் கலந்து பேசினேன். நான் கேள்வி கேட்க அனுமதி இருந்தது. என் கேள்விகளுக்கு திருப்திகரமான பதில்கள் கிடைத்தன. தேவைப்பட்டால் எழுத்து மூலம் பதில்கள் கிடைக்கும் என்பதையும் அறிந்து கொண்டேன். இந்த ஆய்வின் போது ஏதேனும் புதிதாக கண்டுபிடிக்கப்பட்டால் எனக்கு தெரிவிக்கப்படும் என்று அறிவேன். எனது பங்கேற்பு தனிப்பட்ட விருப்பமானது என்பதை அறிவேன்.

எனது தனிப்பட்ட தகவல்கள் பாதுகாப்பாக வைக்கப்படும் என்பதை அறிவேன். ஏதேனும் காரணத்தினால் இந்த ஆய்வில் இருந்து வெளியேற நினைத்தால் அவ்வாறே செய்யலாம் என்றும் இதனால் சிகிச்சைக்கு பாதிப்பு இருக்காது என்பதையும் அறிவேன். ஆய்வின் காரணமாக எனக்கு உடல்நல பாதிப்பு ஏற்படுமானால், இலவச சிகிச்சை அளிக்கப்படும் என்பதை அறிவேன்.

மேற்கூரிய ஆய்வின் சாதக பாதகங்களை நன்கு அறிவேன் என்றும் மேற்கூறிய செயல்முறைகளில் பங்கேற்க முழுமனதுடன் ஒப்புக் கொள்கிறேன் என்றும் உறுதி கூறுகிறேன். கையெழுத்திட்ட ஒப்புதல் படிவ நகல் பெற்றுக் கொண்டேன். எனது பரிசோதனை முடிவுகளும் தனிப்பட்ட தகவல்களும் யாருக்கும் தெரியாதவாறு பாதுகாக்கப்படும் என்பதை நன்கு அறிவேன்.

தேதி : பங்கேற்பாளரின் ஒப்பம் /
இடது விரல் கைரேகை

நான் மேற்கூறிய ஆய்வை பற்றிய முழுமையான விளக்கங்களையும் சாதக பாதகங்களை என்னால் இயன்றவரை .....க்கு எடுத்துரைத்தேன்.

ஆராய்ச்சியாளரின் கையொப்பம் பங்கேற்பவரின் கையொப்பம்

தேதி :

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

EC Reg No.ECR/270/Inst./TN/2013
Telephone No. 044 25305301
Fax : 044 25363970

CERTIFICATE OF APPROVAL

To
Dr. K.Shanmuga Priya,
Postgraduate M.D.(Dermatology, Venereology and Leprosy),
Madras Medical College,
Chennai - 600 003.

Dear Dr.K.Shanmuga Priya,

The Institutional Ethics Committee has considered your request and approved your study titled **"A study on the prevalence, isolation and sensitivity pattern of genital candida species in female patients attending STD outpatient department". No.13102014.**

The following members of Ethics Committee were present in the meeting held on 07.10.2014 conducted at Madras Medical College, Chennai-3.

1. Dr.C.Rajendran, M.D.,	: Chairperson
2. Dr.R.Vimala, M.D., Dean, MMC, Ch-3	: Deputy Chairperson
3. Prof.B.Kalaiselvi, M.D., Vice-Principal, MMC, Ch-3	: Member Secretary
4. Prof.R.Nandhini, M.D., Inst.of Pharmacology, MMC	: Member
5. Prof.K.Ramadevi, Director i/c, Inst.of Biochemistry, MMC	: Member
6. Prof.Saraswathy, M.D., Director, Pathology, MMC, Ch-3	: Member
7. Prof.S.G.Sivachidambaram, M.D., Director i/c, Inst.of Internal Medicine, MMC	: Member
8. Thiru S.Rameshkumar, Administrative Officer	: Lay Person
9. Thiru S.Govindasamy, B.A., B.L.,	: Lawyer
10. Tmt.Arnold Saulina, M.A., MSW.,	: Social Scientist

We approve the proposal to be conducted in its presented form.

The Institutional Ethics Committee expects to be informed about the progress of the study and SAE occurring in the course of the study, any changes in the protocol and patients information/informed consent and asks to be provided a copy of the final report.

15/10/14

Member Secretary, Ethics Committee

MEMBER SECRETARY
INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COL.
CHENNAI-600 003